CASSAVA DROUGHT TOLERANCE MECHANISMS RE-VISITED: EVALUATION OF DROUGHT TOLERANCE IN CONTRASTING CASSAVA GENOTYPES UNDER WATER STRESSED ENVIRONMENTS

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Cassava (*Manihot esculenta* Crantz) is a perennial root crop from the neo-tropics, which is considered a food security crop against famine in drought prone areas. The objectives of this work were to identify traits in cassava that contribute to drought tolerance and evaluate the potential to use these traits in cassava breeding programs. Two sets of cassava genotypes containing 45 and 15 lines were used to assess drought response under field and screen house conditions. Morpho-physiological traits evaluated included sugar and starch contents in leaves and stems, abscisic acid (ABA) accumulation, leaf retention (LR), relative water content (RWC), leaf canopy temperature (T), leaf-air temperature difference (T*d*), leaf chlorophyll greenness (CG) and root growth, among others. In field-grown potted plants at the early phase of water deficit, leaf ABA in the afternoon was positively correlated with soil water content and negatively correlated with T and T*d* in the morning, suggesting that genotypes with stomatal closure in the afternoon conserved water, permitting morning stomatal opening at DAY 30 of water deficit. Storage root weight was not correlated with aboveground fresh biomass (AGB), whereas it was strongly correlated with partitioning index. Broad sense heritability and phenotypic standard deviation were sufficiently high to predict that response to selection would be successful for several traits including PH, T, T*d*, CG, and PI. Genotypes CM 3306-9 and

MBRA 165 ranked high for yield and partitioning index in both environments. Sampling a set of 15 genotypes during the progression of stress onset showed that fibrous roots and stems accumulated ABA at an early stage, while leaf and stem ABA were negatively correlated with carbohydrate accumulations. Leaves in both control and water stress treatments maintained high RWC. Root and stem carbohydrate reserves were gradually depleted during stress. Genotypes previously characterized as stress tolerant had a smaller reduction in biomass when compared to their susceptible counterparts. I conclude that several of the traits examined could be useful in phenotyping genotypes for drought tolerance.

BIOGRAPHICAL SKETCH

Luis Orlando Duque was born in Queens, New York and graduated with a Bachelor of Science in Agronomy (Ingeniería Agronómica in Spanish) from the Universidad Nacional de Colombia –Sede Palmira- in the year 2000. Afterwards, he traveled to the United States to pursue graduate studies. In the year 2003, Luis was awarded a SUNY Minority Fellowship and undertook his graduate studies in the Department of Crop and Soil Science. Afterwards he graduated with a Masters in Science in the year 2007. In that same year, Luis started his doctoral degree in the same department and finished in 2011. Currently, Luis lives with his wife in Ithaca, New York.

Dedico este triunfo a mi gnomita feliz. Sin ti nunca lo hubiera logrado.

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1.1 Introduction

Cassava (*Manihot esculenta* Crantz) represents the difference between survival and famine for over 600 million inhabitants of Africa, Asia and Latin America (IFAD, 2010). It is the third most important source of calories in the human diet in the tropics, behind maize (*Zea mays* L.) and rice (*Oryza sativa* L.), making it an essential source of dietary energy for millions of people worldwide. In sub-Saharan Africa[1]cassava ranks second to maize in source of calories (Sayre et al., 2011). In addition, it provides a livelihood for farmers and industrial sectors of developing economies alike. The distribution of cassava is limited to developing tropical and sub-tropical countries, but the crop has recently been expanding into more marginal lands, particularly in sub-Saharan Africa (Romanoff and Lynam, 1992). In these areas, staple food crops such as maize and rice produce poorly because of rising degradation of these marginal African ecosystems coupled with climate change (e.g., increased CO_2 concentration and/or temperature variation), drought (e.g., erratic distribution and/or decreased precipitation) and

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[2] For the for the purpose of this chapter, I have consciously left out other developing regions of the world such as Latin America and Asia that have also suffered from drought episodes and that have hindered cassava development and production. Of these regions, sub-Saharan Africa is the least developed and the most prone to famine, war and drought. Under this premise, cassava can serve as a food staple and food insurance

inability to supply fertilizer and control pests and weeds due to poverty (e.g. subsistence, smallholder and/or resource-limited farmers) (Cadavid et al., 1998; De Tafur et al., 1997; El-Sharkawy, 1993; Florchinger et al., 2000). Over 40% of cassava production in the world occurs in sub-Saharan Africa, where it is commonly grown by resource-limited farmers on marginal and highly eroded low-fertility soils without the application of agrochemicals (El-Sharkawy, 1993; El-Sharkawy, 2004; Ruppenthal et al., 1997). Cassava's ability to yield comparatively well in poor soils and environments with abiotic and biotic stresses is particularly appealing to small farmers throughout the tropics, especially sub-Saharan Africa. Its broad agro-ecological adaptability, inherent drought tolerance and capacity to produce reasonable yields in adverse conditions establishes cassava as a foundation crop for food security at a household level. These attributes combined with socio-economic considerations have made cassava a leading crop in poverty alleviation particularly in sub-Saharan Africa.

Climate change has significantly impacted the African continent through increased frequency of drought episodes and unpredictable and erratic rainfall patterns (Jama and Pizarro, 2008; Rosenzweig and Parry, 1994). Over 40% of the population lives in arid, semi-arid, or dry sub-humid areas, making Africa one of the most susceptible areas of the world to global warming (Chhibber and Laajaj, 2008). Specifically, sub-Saharan Africa is thought to be the region most vulnerable to the impacts of climate variability and change because agriculture plays a dominant role in supporting rural livelihoods and economic growth over most of this region (Challinor et al., 2007). But the rural poor of sub-Saharan Africa rely to a large extent on rain-fed agriculture or pastoralism for their subsistence. Such communities, which are already struggling to cope with the impacts of current climatic variability, will face overwhelming tasks in adapting to future climate change. Climate models indicate that sub-Saharan Africa will be drier and

substantially hotter than at present (Battisti and Naylor, 2009), which leads to increased evapotranspiration potential and the potential for drought (Houghton, 2005). In sub-Saharan Africa, drought tolerant crops will remain vital for food security (Cooper et al., 2008). Drought-prone areas are one of the most important types of marginal environments, because drought is a principal direct cause of crop failure and economic losses to farmers (Fuglie, 2007; Kosina et al., 2007; Pandey et al., 2007). Low yields in sub-Saharan Africa are strongly linked to water availability for crop production, which constitutes a main constraint to food security for millions of smallholder farmers in this region (Falkenmark and Rockstrom, 2008). In addition, limited crop water availability in combination with a range of other constraints (e.g., to poor soils, insect pests, and lack of inputs and labor) leads to decreased yields in staple crops such as maize and rice (Falkenmark and Rockstrom, 2008). This makes small-scale farming a highly uncertain food and income source for sub-Saharan Africa (Conway and Toenniessen, 2003). Thus, drought is a recurring reality in many parts of Sub-Saharan Africa, where rain-fed agriculture continues to be a major sector of most economies. Moreover, there is increasing scientific evidence indicating that if current climate trends continue, drier areas will become drier and droughts more frequent (Jama and Pizarro, 2008; Rosenzweig and Parry, 1994). Since drought is an important contributor to yield reduction of staple crops in sub-Saharan Africa, food security has declined substantially during stressed years contributing to more poverty in this region.

Though poverty is a complex issue for many sub-Saharan countries, environmentally speaking, soil degradation, increasing water scarcity, poor management of water, desertification, and climate change are considered the major components in the exacerbation of this crisis.

Famine and food insecurity are inextricably linked to poverty in Africa. Thus, these two problems are of great concern in reference to basic development issues impeding national

economic growth and are a major part of what keeps millions trapped in poverty (African-Union, 2005). In the same way, climate change, i.e., weather risks contributes to low investment and hence to long-run agricultural stagnation and rural poverty in countries that depend on rainfed agriculture (Stephen, 2007). The Millennium Development Goals (MDGs) were established by the 192 United Nations member states and several international organizations in the year 2000. The MDGs consist of eight international development goals such as erradiacating extreme poverty and hunger, reducing child mortality rates, ensuring environmental sustainability, etc. that should be achieved by the year 2015. The semi-arid and dry sub-humid regions of sub-Saharan Africa present large challenges in terms of the Millennium Development Goal of eradicating poverty and hunger. It is estimated that 45 to 50% of the population live in extreme poverty and the level of malnutrition has increased (UNDP, 2006).

What is more, the World Bank (2006) states that 83% of the poor live in rural areas and only 4% of farmed areas have access to irrigation. The livelihoods of the poor in addition to the economies of most developing countries that depend on agriculture are highly impacted by rainfall variability (Gautam, 2006).

Evidence has shown that without a solid and emergent agricultural sector it is problematic to decrease poverty and eliminate food insecurity. Hence, research strategies to address increasing poverty in such regions must focus on raising productivity, profitability and sustainability, given the importance of agricultural activity in food security and livelihoods of the poor. (Dixon et al., 2001; Gryseels et al., 1992). Researchers worldwide have a growing concern that development efforts should focus on accelerating progress in reducing poverty and hunger of resource-poor farmers in marginal environments. Therefore, poverty reduction will be exceedingly difficult in sub-Saharan Africa exclusive of improved agricultural growth. Poverty

reduction and increased food security in sub-Saharan Africa depend on agricultural development. Only enhanced agricultural throughput can concurrently advance welfare amid the two-thirds of all Africans who work mainly in agriculture as well as the urban poor who devote over 60 percent of their budget on food staples. Therefore, it is up to the shared capacity of African farmers, governments, and agricultural researchers to encourage and support comprehensive agricultural growth in order to produce meaningful decreases in poverty. There is ample evidence that significant outcomes in poverty reduction can result from fructiferous smallholder farming and participation in competitive markets when offered the needed assistance.

Climate prediction models indicate considerable decrease of yield of many important staple crops, decrease in food security and poverty due to agricultural stagnation in sub-Saharan Africa as a result of warmer and drier climate change events.(Kamukondiwa, 1996; Makadho, 1992). One of the key factors for poverty alleviation and sustainable development in developing countries is through sound agricultural research and implementation. Thus, sustainable agriculture under a warmer and drier climate will require proper choice of plants with high water-use efficiency (WUE), capacity to maintain production at high ambient CO_2 concentrations, drought tolerance and overall sustainable yield (Rosenzweig and Parry, 1994).

Recent research has shown that cassava is a prime example of a staple crop that initially was undermined by agricultural developmental policies in addition to receiving considerably less emphasis in crop improvement and biotechnology when compared to maize or rice. Industrial agricultural producers have also neglected the crop because of its past reputation for low profitability, even though cassava is improving the livelihood of millions throughout sub-Saharan Africa. Nonetheless and despite cassava's importance as a staple crop , and its contribution to fighting hunger and poverty in developing countries, and despite successful

transitions toward use of cassava starch in production of processed foods and industrial materials in Thailand and Brazil, cassava is still neglected in agricultural development policies and technologies. In addition, cassava is often relegated to marginal lands due to competition with higher-value crops, such as rice and maize. This trend is likely to continue as such crops are further improved to adapt them to marginal conditions. Cassava has undergone many conventional breeding efforts, which have attempted to address many of the productivity constraints, but with limited success. Progress has been sluggish because of the crop's complex genetic makeup, which makes it problematic to breed efficiently. In addition, cassava is vegetatively propagated through stakes and not through true seed, which makes cassava's seed stock highly perishable, and difficult to manage and transport. Hence, to develop cassava lines that have performance that lives up to its full potential will require a fairly substantial investment. Over time, cassava often has wound up in hill-lands, lands with low soil fertility, or lands susceptible to periodic or seasonal drought or flooding (Plucknett et al., 1998).

The main objective of this chapter is to present an overview of the potential of cassava as a major staple food crop by asserting its intrinsic importance in alleviating food insecurity while at the same time contributing to poverty alleviation and socio-economic development in sub-Saharan Africa, in part due to its resilience to marginal and adverse environments. At the outset, the chapter will briefly focus on the origins and distribution of cassava throughout history and in sub-Saharan Africa[2]. Second, it will define what makes a potential and successful staple food crop (e.g. a "king" crop) and analyze if cassava fulfills the requirements of converting itself from a "peasant" crop to a "king" crop. I will then, examine cassava's impact

[2] For the for the purpose of this chapter, I have consciously left out other developing regions of the world such as Latin America and Asia that have also suffered from drought episodes and that have hindered cassava development and production. Of these regions, sub-Saharan Africa is the least developed and the most prone to famine, war and drought. Under this premise, cassava can serve as a food staple and food insurance under these conditions. In addition, it has been well documented that cassava is emerging as an important industrial crop for bio-fuel production and a main source of industrial starch, however, these topics are not considered here.

on socio-economic development as a staple crop throughout sub-Saharan Africa. Lastly, this chapter summarizes key findings, considerations and recommendations for the future of cassava as a leading staple crop for subsistence farmers, traders, and industry.

1.2 Distribution, origins, domestication and history

The genus *Manihot* includes approximately 98 species distributed throughout the New World tropics, from Mexico to Argentina. Most species occur in northern South America (~80 species), and there is a secondary center of species diversity in Central America and Mexico (~17 species) (Olsen, 2004; Olsen and Schaal, 2006; Schaal et al., 2006).

Numerous studies have dealt with the origin of domesticated cassava (Allem, 1999; Sauer, 1993), but the most conclusive study to date was reported by Schaal et al. (2006), who used SNP (single nucleotide polymorphism) and SSR (simple sequence repeat) variation analyses as a means of tracing cassava's evolutionary and geographical origins. Their evidence suggests that cassava was likely domesticated in the southern Amazon basin from a single wild *Manihot* species, *M. esculenta ssp. flabellifolia*, rather than from multiple hybridizing species as traditionally believed.

Determining the wild ancestor of cultivated cassava allowed researchers to further inquire into the morphological changes associated with domestication. Schaal et al. (2006) determined that there are three morphological distinctions between *M. esculenta* ssp. *flabellifolia* and modern cassava cultivars. Their analysis helped identify traits that have been altered during domestication. First, subspecies *flabellifolia* can grow to a height of 7 meters and present a

diameter breast height up to 20 cm in a long-lived stand, whereas modern cassava is relatively short. But this subspecies is highly plastic. If the surrounding forest is cleared, *M. esculenta* ssp. *flabellifolia* will grow as a short and erect shrub-like plant similar to that of the modern cassava plant. A second change associated with domestication is the development of a fleshy storage root high in starch. This is the most noticeable difference between the ancestor and the cultivated plant. Lastly, the vegetative propagation of the species is remarkably different. *M. esculenta* ssp. *flabellifolia* flowers freely, whereas flowering in the modern cassava cultivars is limited with few flowers and partial fruit set. This specific condition implies a long history of vegetative propagation in which modern cassava cultivars have differed from *flabellifolia* in the trade-off between sexual and asexual reproduction (Schaal et al., 2006).

The origin of cassava in Africa has been studied by numerous authors (Jameson and Thomas, 1970; Jennings, 1976; Jones, 1959; Purseglove, 1968). Portuguese sailors navigating from Brazil during the slave trade introduced cassava into the west coast of Africa in the 16th century. Written sources first mention cassava cultivation in the Portuguese outposts on the Angolan coast in 1608 (*Loango*) and in the 1620s (around *Luanda*). Subsequently, traders moved cassava into east Africa, specifically towards Madagascar and Zanzibar (Jennings, 1976). Other historical accounts show that cassava was taken from Brazil to Reunion, off the coast of eastern Africa in 1736 and it was recorded in Zanzibar in 1799 (Purseglove, 1968). However, other researchers (Von Oppen, 1991) have cited records indicating that central Africa adopted cassava much more rapidly than coastal regions. These finding suggest that cassava cultivation began before the mid-sixteenth century during the *Luunda* Empire in what is now known as southwestern Zaire.

The first appearance of cassava, nevertheless, should not be perceived as a process that

happened all of a sudden. Rather, it is assumed to be a lengthy progression of importation and testing with adoption of some of the newer varieties, while others were rejected (Von Oppen, 1991). Nearly all of the dissemination of cassava in Africa inland and along riverside markets began during the 20th century due to the colonial reinforcement of its planting as a backup against famine and the ability to counter pests. Currently, cassava is grown in several African countries from south of the Sahara desert to north of the Limpopo River (Hillocks, 2002)

1.3 What makes a crop a "king" crop

It is estimated that the world's population will reach approximately 6.79 billion in 2009 and will continue to grow to around 9.5 billion by the year 2050 (USCB, 2008). Although the population growth rate has been steadily declining from its peak of 2.19% in 1963, growth remains high in Latin America, the Middle East and Sub-Saharan Africa (Nielsen, 2006). Specifically, the population of sub-Saharan Africa has been estimated at 860 million in 2009 with a current growth rate of 2.3%. The UN predicts for the region a population of nearly 1.5 billion in 2050 (UN-ESA, 2008). Sub-Saharan Africa is considered the poorest region in the world, suffering from the effects of economic mismanagement, local corruption, inter-ethnic conflict and unpredictable biotic (e.g., diseases) and abiotic (e.g., drought) stresses. The public sector expects agricultural science to respond to increases in population, poverty and food insecurity, by providing technologies that will maintain and increase production.

One of the key factors for poverty alleviation and sustainable development in developing countries is sound agricultural research and implementation. Thus, agriculture is considered a reliable medium to reduce rural poverty. In most developing countries, agriculture and agriculture-related activities provide most of the employment in rural areas (Machethe, 2004).

Rural people use natural resources – land, water, and biotic resources – as the base of their livelihood. These resources are dominant factors of production in agriculture, the major economic activity in rural areas of sub-Saharan Africa.

One important and key element for the success of agriculture is the crop in and of itself. Thus, for a crop to be successful it has to be adaptable, adoptable, sustainable and profitable for farmers and industry alike. Resource-limited farmers in sub-Saharan Africa have to constantly decide what crops to adopt depending on their resistance to biotic and/or abiotic stress and take into account their yield stability throughout the growing season at any given location. In addition, yield stability has to endure for many seasons, offering continuous food security, sustainability, and peace of mind to the farmer. Subsequently, if yields are sustainable and have potential to increase with better agronomic practices, yield surpluses can be traded or sold for a profit.

1.3.1 Crop adaptability

Plant scientists have defined *stress adaptation* as a genetically determined level of resistance or tolerance acquired by a process of selection over many generations. Adaptation to biotic or abiotic stresses results from integrated processes at all levels of organization, from the anatomical and morphological level to the cellular, biochemical and molecular level (Taiz and Zeiger, 2002). Types of abiotic stresses encompass: water deficit, salinity, chilling and freezing, heat, and oxygen deficiency among others. Crop adaptation is an important factor to take into account for the development of sustainable agricultural systems. Broad and/or specific adaptations are important features of genotypes allowing for a better use of the available natural resources. Understanding the causes and nature of adaptation can help in making better

predictions for genotypic performance in changing agricultural scenarios, and increasing the efficiency of selection processes for new genotypes. For plant breeders, plant adaptation to the target environments for which cultivars are developed is a fundamental process. Adaptation of plants to cultivated environments was the first criterion in the initial domestication of plants thousands of years ago. Adaptation is generally a quantitative complex feature of plants, involving many traits, many of which are genetically quantitative. Adaptations to abiotic stresses like cold or drought or abiotic stresses such as diseases are among the most central problems in a world grappling with global food security.

Modern plant breeding, based on Mendelian genetics, has made plant improvement more effective, precise, and selective. Molecular genetics and genetic engineering has considerably increased this selectivity down to single genes affecting single traits. However, the efficiency of plant breeding may cause loss of useful untapped genes, reduction of biodiversity and a lesser adaptation to a wider range of environments through monoculture practices. In a well designed plant breeding program, an effort is made to merge modern plant breeding efficiency with ecological aspects of plant breeding, reflected in environmental adaptation. It is hoped that this merger results in more sustainable use of genetic resources and physical environments.However, target environments as well as societies' goals can also alter both dynamically and unpredictably, rendering once useful genetic resources useless. In a world where climates and environments are under continuous change and where human society is increasingly polarized into a developed and a developing part, adaptation of our cultivated plants has different constraints on yields depending on the ecology of the target environment and economy of the target society (Tigerstedt, 1996.).

Crop adaptation strategies may be used to reduce losses or increase gains in light of

potential impacts of climate change (Giannakopoulos et al., 2005). In general, agronomic adaptation in the temperate zone is projected to ameliorate crop yield losses due to environmental stresses and improve gains. However, in tropical areas where temperatures are already high and pest pressure is more severe, there may be need for altogether different improvement strategies tailored for increased and sustained crop yields for the resource-poor farmer. In the short term, crop systems can be managed to reduce yield variability by utilizing already-developed stress tolerant crop varieties. In the long-term, agricultural adjustments involve changes to the larger structural system to optimize production, including changing land allocation, developing newer cultivars that are drought tolerant and possess higher water use efficiency coupled with sound agronomic practices.

1.3.2 Crop adoptability

Agriculture can be characterized according to specific environmental, farmer behavioral, and policy aspects. Many of the problems that have resulted in limited benefit from technological advances are associated with misguided agricultural development such that the products of agricultural research are not adopted by farmers (Sanzidur Rahman, 2005). However, the environment has played an important part in how newer technologies are implemented and adopted throughout the developing world. Thus, appropriate strategies to harness potential benefits of improved varieties in diverse agro-ecological and socio-economic environments are essential. The lack of adequate information on farmers' perception and adoption regarding new varieties has often placed them in the wrong target regions where they either failed or are met with partial success.

Although agricultural research has accomplished massive benefits for farmers and

consumers in developing countries around the world many have been excluded from these benefits (Bellon, 2006; Evenson and Gollin, 2003; Pingali, 2001). Several reasons attest to the fact that mistargeted agricultural development and low rates of crop adoption have limited the extent to which subsistence farmers in developing countries benefit from agricultural development. Bellon (2006), points out four major reasons for this phenomenon: 1) these farmers are located in marginal lands coupled with adverse environments, 2) many of these farmers are indigenous or from socially-marginalized groups with no voice or political power in their countries, 3) population distribution is erratic, being more concentrated in the cities and less dense in the rural areas and, 4) agricultural research has focused primarily on a few staple crops of global importance such as maize, rice, and wheat. Thus, agricultural research should be designed such that the likelihood of adoption by needy subsistence farmers is enhanced and the benefits will make important contributions to poverty alleviation, food security, and environmental sustainability (Bellon, 2006).

Two major factors affecting the rate of adoption of rural innovation are *risk* and *uncertainty of outcome*, though empirical evidence that refutes or agrees with this has been scarce(Abadi Ghadim et al., 2005). Abadi Ghadim et al. (2005) explains this phenomenon as the "*practical difficulty of obtaining high quality measures of the relevant variables*". Therefore, appropriate economic risk variables with likely impacts on adoption of innovations would include: 1) farmers' perceptions of the riskiness of the innovation, 2) farmers' uncertainty about the innovation, 3) farmers' potential to learn about risk and reduce uncertainty through testing the innovation; and 4) farmers' attitudes to risk and uncertainty.

Resource-poor farmers in marginal areas confront numerous risks associated with their surrounding environment and have limited capacity to cope with them (Bellon, 2006). Important

natural risks include drought, pests and diseases, but also risk can be associated with the storage and marketing of their products. Hence, it is essential to understand the risks that these farmers face and their ability to manage them in order to generate research products that have improved risk profiles compared to their current options.

1.3.3 Crop sustainability

The concept of sustainable crop production aims to produce food in adequate quantity and quality, whilst maintaining or improving the surrounding environment and biodiversity. However, intensive management of agro-ecological systems has in many cases reduced biodiversity and undermined ecosystem functions. Sustainable agriculture integrates three main goals: environmental health for the ecosystem or area in question, economic profitability for the farmer, and social and economic equity for the farming community (UC-SAREP, 1997).

Regarding environmental health, sustainable agricultural practices involve a variety of approaches, though relevant to the objective of this chapter is the selection of genotypes and varieties that are well suited to the specific agroecological niche. Of most importance is the proper choice of a crop that can thrive and endure in marginal ecosystems such as in areas with intermittent and erratic precipitation coupled with long dry spells, low soil fertility, improper infrastructure and lack of socioeconomic development. In addition, other specific strategies must take into account topography, soil characteristics, climate, pests, local availability of inputs, and the individual farmer's goals. Thus, crop sustainability is dependent on, affected by and a function of several intrinsic factors, which properly understood and implemented can sustain the economic viability for smallholder peasant farming.

1.3.4 Crop profitability

Recent findings demonstrate that the fate of the world's most impoverished depends on their countries' and regions' performance in agricultural and rural development (Binswanger, 2006). Given the finite supply of natural resources at any specific cost and location, agricultural activity and production should be efficient in its use of limited natural resources, such as water supply and mineral nutrients, and should not damage or exhaust them.

In agriculture, cash crops are crops that are grown for a profit, a term used to differentiate them from subsistence crops, which are those grown and utilized to feed the producer's family. In numerous agroecological systems there is a competition between growing subsistence crops and cash crops. In general, resource-limited farmers on marginal lands are paid for their cash crops, but if a natural disaster occurs, such as a prolonged drought, cash crop production can fall sharply resulting in decreased or no yield, in addition to lower money paid for the produce, exacerbating food insecurity and enhancing poverty. On the other hand, subsistence crops (i.e., crops grown as food insurance against famine) are generally defined as those that are resilient to environmental change, adapted to marginal lands, and require low purchased inputs, resulting in acceptable and sustainable yields throughout difficult years.

Yet many controversies exist on the adoption and utilization of cash crops or subsistence crops depending on added value and environmental conditions. Decisions to plant cash crops can be linked to financial aspirations and changing environmental circumstances (Finnis, 2006). Little and Horowitz (1987)argue that decisions about what crops to cultivate should be left to individual farmers, to capitalize on the perceived advantages of different crops.

1.4 Is cassava fit to be "king"?

"Cassava is to the African peasant farmers what rice is to the Asian farmers, or what wheat and potato are to the European farmers." (Alfred Dixon, cassava breeder, IITA)

Currently, about half of cassava's world production lies in sub-Saharan Africa. Cassava is cultivated in around 40 African countries, stretching through a wide belt from Madagascar in the Southeast to Senegal and to Cape Verde in the Northwest. Around 70 percent of Africa's cassava output is harvested in Nigeria, the Congo and Tanzania (IFAD, 2001). Throughout the forest and transition zones of Africa, cassava is either a primary staple or a secondary food staple.

According to the World Bank, the average 2002 per capita GDP in sub-Saharan Africa was $577 or, in other words, less than $2 per day. Approximately three quarters of the poor in Africa are rural people who secure their livelihood from agriculture. Sub-Saharan Africa's population is expected to double to 1.5 billion in 2050, and its urban population will grow at an even faster rate (McCalla, 1999).

Domestic food production and/or food imports will have to be increased to meet sub-Saharan Africa's growing food demand. Due to poverty and a lack of foreign exchange, Africa's net cereal imports are expected to remain low (Pinstrup-Andersen, 2000). Hence, it is of prime importance that sub-Saharan African nations increase domestic food production.

The most important staple food crop for Africa is maize and it is considered a model commodity for meeting the demand of Africa's increasing urban population for suitable foodstuffs. (Blackie, 1990; Byerlee and Eicher, 1997; Mellor et al., 1987). Nevertheless, maize production is unpredictable due to erratic climatic factors such as drought. Numerous studies have shown that cassava is the third major carbohydrate source for human comsumption in the

world. For sub-Saharan Africa, cassava is the second most important food staple in terms of per capita calories consumed (Sayre et al., 2011).

Cassava has the potential to increase farm incomes, reduce rural and urban poverty and alleviate food insecurity. Cassava is vegetatively propagated and can be produced on marginal lands with low purchased inputs in addition to offering flexibility in the timing of labor inputs and harvesting. This flexibility makes cassava particularly attractive to labor-deficit and HIV/AIDS households, making it attractive and a low risk crop for the resource poor. Also, cassava is available to low-income rural households in the form of simple food products (for example, dried roots and leaves), which are less expensive per kg dry weight than grains such as rice, maize, and wheat (Nweke et al., 2001). Cassava has several other advantages over rice, maize and other grains as a food staple in areas where there is a degraded resource base, drought, and weak market infrastructure. In general terms, cassava is considered to be drought tolerant, an attribute that makes it suitable during dry spells and famine. Cassava has historically played an important famine-prevention role in Eastern and Southern Africa where maize is the preferred food staple and drought is a recurrent problem (Nweke et al., 2001). In an ethnographic analysis, Romanoff and Lynam (1992) corroborate that famine rarely occurs in areas where cassava is grown widely because it provides a stable base to the food production system.

Fynn and Haggblade (2006) contends that the reduction in poverty over the past decade has been driven by the combination of growth of increasingly important food crops such as cassava, sweet potatoes (*Ipomoea batatas)*, and groundnuts (*Arachis hypogaea*) which have helped to buoy rural incomes despite the decline in maize production and the well-documented political, economic and environmental negative shocks affecting rural livelihoods.

Conversely, cassava research by the international scientific community has been neglected or at least is not comparable to major staple crops like maize, rice, and wheat (*Triticum* ssp). Often, cassava has been marginalized in food policy debates and burdened with the stigma of being an inferior crop (i.e., "poor man's crop", "orphan crop", "peasant crop", etc.).

Many food policy analysts consider cassava as an inferior quality staple food or Giffen[3] good whose demand is driven by poverty that makes their consumers/purchasers unable to afford superior food products. As the price of the cheap staple food rises, low-resource consumers can no longer afford to supplement their diet with better foods, and must consume more of the inferior staple food and it is assumed that its per capita consumption will decline with increasing per capita incomes.

Presently, cassava research and development has increased and impacted agricultural development throughout the world in the few last years (Ortiz, 2007). A study by (Maredia and Raitzer, 2006) suggested that the main developmental effect of the Consultative Group on International Agricultural Research (CGIAR) centers in sub-Saharan Africa originated because of the backing of long-term crop improvement for yield accompanied with environmental adaptation and integrated pest management research that deal with biotic stress such as insects and diseases. Certainly, the International Institute for Tropical Agriculture (IITA) focused its research on increasing yields in a wide range of agro-ecological zones and agricultural systems suited for diverse consumer preferences through use and implementation of genetically modified cassava genotypes in many African countries. There were about 206 releases of cassava cultivars

[3] A Giffen good is one which people paradoxically consume more of as the price rises, violating the law of demand. The classic example given by Alfred Marshall in this book The Principles of Economics (1890) is of inferior quality staple foods, whose demand is driven by poverty that makes their purchasers unable to afford superior foodstuffs. As the price of the cheap staple rises, they can no longer afford to supplement their diet with better foods, and must consume more of the staple food.

in 20 African nations between 1970 and 1998. In the 1990s African programs incorporated IITA-bred materials in 80% of their cassava breeding progenies, that led to 50% gains in cassava yields on average (Manyong et al., 2000). The above mentioned improved cassava genotypes raised per capita output by 10% continent-wide, benefiting 14 million farmers and therefore embodied a critical impact to Africa's food security, mainly among the poor (Nweke, 2004; Nweke et al., 2001). For example, partnerships between the National Agriculture Research Organization (NARO, Uganda) and the IITA yielded benefits of nearly US $ 36 million over four years (1998-2001) stemming from an initial investment of US $ 0.8 million through the multiplication partnership project to fight against the cassava mosaic disease pandemic in six districts (Dixon and Ssemakula, 2008). The achievements of cassava research in sub-Saharan Africa suggest the advantages of having an international research center such as IITA with crop breeding responsibilities in collaboration with many public and private partners, delivering new technological advances such as improved cassava seed that have a positive impact on African livelihoods (Ortiz and Hartmann, 2003). Undoubtedly, there are certain situations where national programs are now adequately established to achieve this responsibility. Thus, international centers have an obligation to quickly convey these accomplishments to national institutes through technology and services (Ortiz and Crouch, 2007).

The adaptability of a crop lies in its capacity to make good use of environmental variations. Performance stability is despite the capacity of the material for highly predictable behavior related to environmental variation. Adaptability and stability are characteristics of the cultivar and allow it tolerate the environment limiting factors and respond to the favorable factors (Borem and Milach, 1998).

Cassava's adaptability relies on its broad agroecological adjustment to different

environments, which includes a wide range of climatic and edaphic conditions including tolerance to drought and to several pests and diseases, thus conferring an advantage under conditions of famine when compared to other staple crops.

Hereof, cassava seems as an important crop opportunity for marginal environments (e.g., drought prone locations), where cereals and other species do not perform well (Nassar and Ortiz, 2007; Ortiz and Hartmann, 2003). For example, Barratt et. al. (2006) have found that in southern Zambia newly developed drought-resistant cassava genotypes perform more stably than maize and thereby offer more food security in this drought-prone area.

The tolerance of cassava to drought is thought to be due to several physiological mechanisms that enable the crop to endure prolonged periods (four to seven months) without precipitation, frequently coupled with high evaporative demands. These mechanisms include: 1) partial stomatal closure in dry air and nearly complete stomatal closure during soil water deficit such that attached leaves do not suffer damage to their photosynthetic system and retain activity for photosynthesis when water supply permits partial stomatal opening (Calatayud et al., 2000; El-Sharkawy, 2004; Itani et al., 1999), 2) abscission of lower canopy leaves to limit transpirational surface area, while retaining at least some photosynthetically competent upper canopy leaves (Duque and Setter, 2005; El-Sharkawy et al., 1992; Lenis et al., 2006), 3) ability to grow roots into deep soil zones during its long period of growth and extract deep soil water that is not accessed by other crops (Duque, 2007; Pardales and Yamauchi, 2003; Tscherning et al., 1995), and 4) drastic reduction in leaf area growth (Alves and Setter, 2000; Alves and Setter, 2004b; Han et al., 2001; Itani et al., 1999). These mechanisms primarily involve water conservation and limited use of carbohydrate such that scarce resources are conserved. Other studies have shown that osmotic adjustment (OA) and accumulation of dehydrin-family LEAs, a

class of desiccation protectant proteins, is minimally used by cassava, at least in short-term stresses of six to eight days (Alves and Setter, 2000; Alves and Setter, 2004a; Han et al., 2001). Recently, it has been shown that substantial amounts of starch are stored in stems and petioles and are remobilized during a drought stress episode, providing a source of carbohydrate for continued metabolic activity (Duque and Setter, 2005).

However, the adoption of improved drought tolerant cassava cultivars depends entirely on the farmer and the willingness to test and continue to grow these new varieties. Adoption increases with factual yield differences between cassava varieties when they are planted in farmers' own fields using their own level of crop management with little or no need for newer inputs (Akoroda and Ikpi, 1988).

With collaboration from the IITA, the continuing adoption rate was first observed on a farmer-to-farmer basis and then extended to other countries. Subsequently, improved varieties were multiplied and distributed among farmers in Nigeria thanks to the National Seed Service (NSS) and the National Accelerated Food Production Program (NAFPP). Conversely, an added incentive in adoption rates by farmers in sub-Saharan Africa was achieved by the awareness of African governments' push for rapid multiplication and distribution using IITA-improved cassava varieties.

Following a CGIAR report from 1996, the IITA indicated that several African countries are on a favorable path. For example, in Nigeria both farmers and the government are taking benefit of the proximity of the IITA and adapting newer technologies for cassava utilization and production. Currently, Nigeria is ranked first as the world's largest producer of cassava above Brazil, Thailand and Zaire (Phillips et al., 2004). This accomplishment, in agreement with FAO, is essentially due to the readiness of improved varieties from IITA.

Thanks to the Nigerian government, there has been an encouraging atmosphere for the growth and coverage of cassava. In 1986, a ban was placed against the importation of maize, rice, and wheat by the Nigerian government in order to promote and increase local food production through the Structural Adjustment Program (SAP). Concurrently, the government implemented assertive and encouraging advertisements to disseminate improved cassava varieties, insisting all pertinent national institutions to set out on the multiplication and distribution of cassava planting materials in the rural areas.

For low resource farmers, cassava has proven to be sustainable and profitable because it does not require purchased inputs. And because of cassava's flexible planting and harvesting schedule, households are able to attend other responsibilities, making it an easy crop for labor compelled ill-disposed households to cultivate. Evidence from Zambia indicates that HIV/AIDS predominance constitutes a slight but statistically significant impact to the expansion of cassava amid affected households (Sitko, 2008). Research indicates the cassava yields can be maintained for over 30 years within the same plot and without fertilizer (Nweke, 2004).

Sub-Saharan African resource-poor farmers and producer groups are the primary beneficiaries who will benefit from the improved ability to manage their production systems for greater and secure productivity, sustainability, and profitability. Also, the availability of a wider array of marketable cassava products; establishment of local employment opportunities thus avoiding migration to urban areas and more important, the alleviation of food insecurity and improvement of rural livelihood coupled with sustainable production environments. Subsequently, the secondary beneficiaries would include small to medium-scale processors, traders, consumers and agribusiness entrepreneurs involved in cassava production, processing, consuming, and marketing who will benefit through boosted cassava supply, demand and

commercialization.

An advantage of cassava is that a farmer is not making an either/or choice between cash crop versus subsistence crop, because cassava is excellent for both. Cassava has good performance in marginal environments where sustainability is an issue, but it also is a very good performer in high input conditions, such as those in Nigeria, Brazil, Thailand, and Indonesia, where cassava is grown as a cash crop (FAOSTAT, 2011). This means that cassava can be grown with the assurance that in a disaster year a farmer can have enough to subsist, while in a good year, cassava production will be high enough to provide cash. To a considerable extent this avoids the debates about cash crop profitability versus subsistence crops that have been described.

Since 1961, cassava production has more than tripled in Africa increasing from 33 million tons per year to 101 million tons fresh weight. By 2020 cassava productivity is expected to double (Scott et al., 2000), thus future trends imply a stable growth over time. For example, in Africa growth rates of 2.5% between 1961 and 1975 and 2.7% between 1976 and 1998 were recorded. In addition, a rate of 4.4% was documented between 1976 and 1998 in West Africa considered one of the major cassava-growing areas (FAO, 2000). Over 60% of the increase was due to the expansion of cultivated cassava and the residual percentage was the effect of increased yields from new improved varieties (Manyong et al., 2000).

Until recently Nigeria superseded Brazil as the world's leading cassava producer. This is due to international, regional, and national research and extension program partnerships within nations. Through public-private entrepreneurship innovations the productivity gains of the new biological and processing technologies have resulted in increased returns to land for farmers when compared to traditional methods. Because of this, consumer prices have fallen for

processed cassava products, contributing to food security both in Nigeria and in Ghana.

Increases in production in countries such as Nigeria and Ghana resulted because of the selection of higher-yielding varieties and improved management practices. Promoting cassava production and utilization presents several challenges. Cassava presents a prolonged vegetative stage (>1 year), with varied production levels dependent on genotype characteristics. Furthermore, cassava is highly perishable and bulky after harvest thus it requires initial processing to lower down transportation costs.

The main growers of cassava in sub-Saharan Africa are low resource subsistence farmers and women are generally responsible in transforming and processing cassava into *gari, fufu, and tapioca*, which is how cassava is consumed within the household. In addition, other value-added cassava products can include chips, pellets, flour, alcohol, and starch. Nowadays the cassava industry produces livestock feed, textiles, confections, plywood, and soft drinks.

However, in Western and Central Africa many developmental objectives have focused in the improvement of farmer's yield and agricultural practices. According to IFAD (2001), increased yield have not translated into increased incomes. Both producers and processors depend on a steady supply of cassava for income and find important the responsibility of efficient markets and value chains through coordination and collaboration.

"Africa's cassava transformation has arguably proven to be its most powerful poverty fighter to date." (Haggblade, 2004)

Many benefits have been revealed through cassava research collaborations between African nations. For example, the distribution of genetic material from IITA and CIAT to

different national programs and between African nations has proven critical in alleviating famines and increasing yield gains. Many African countries that share similar agroecological zones have facilitated and benefitted from joint collaborations through movement of cassava germplasm over the past decades.

Efforts by cassava scientists across Africa have countered major biotic and abiotic stresses and have converted these threats into opportunities for significant subsequent rapid production growth, benefiting tens of millions of small farmers and making cassava one of the continent's most powerful poverty fighters to date, sustaining its food security role.

Cassava's potential productivity could become the essential base for an array of processed products that will effectively increase demand for cassava and contribute to agricultural transformation and economic growth in developing countries. To achieve this, scientists and economists alike can work together to determine the best ways to link supply and demand, strengthen the integration of participants operating within the cassava chains, increase the added value of processed cassava roots, and open up new market outlets for cassava derivatives.

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CHAPTER 2

Response of several morpho-physiological traits to early drought in cassava and their association with drought tolerance

2.1 Introduction

Drought is one of the most important environmental stresses in agriculture and understanding the physiological mechanisms of drought tolerance that are the most valuable for crop production is a major goal of agricultural researchers (Cattivelli et al., 2008; Richards, 2006). In many tropical and sub-tropical countries of the world, the climate involves alternate rainy and dry seasons, where the amount and timing of rains varies substantially from year-to-year. In these climates, the ability to produce a crop that survives severe water deficit and maintains plant development under intermittent or terminal drought conditions may allow the production of yields that contribute to the food security of subsistence farmers and to the economy of the local population. The ideal outcome for all farmers is to grow plants that can survive and maintain relatively high yields regardless of drought conditions (Neumann, 2008).

Cassava is a staple crop for many tropical drought-prone areas and it contributes to food security against famine due to its inherent drought tolerance (El-Sharkawy, 2004; El-Sharkawy, 2006; Setter and Fregene, 2007). The tolerance of cassava to drought is thought to be due to several physiological mechanisms that enable the crop to endure prolonged periods (four to seven months) without precipitation, frequently coupled with high evaporative demands. These mechanisms include: 1) partial stomatal closure in dry air and nearly complete stomatal closure

during soil water deficit such that attached leaves do not suffer damage to their photosynthetic system and retain activity for photosynthesis when water supply permits partial stomatal opening (Calatayud et al., 2000; El-Sharkawy, 2004; Itani et al., 1999), 2) abscission of lower canopy leaves to limit transpirational surface area, while retaining at least some photosynthetically competent upper canopy leaves(Duque and Setter, 2005; El-Sharkawy et al., 1992b; Lenis et al., 2006), 3) ability to grow roots into deep soil zones during its long period of growth and extract deep soil water that is not accessed by other crops (Duque, 2007; Pardales and Yamauchi, 2003; Tscherning et al., 1995), and 4) drastic reduction in leaf area growth (Alves and Setter, 2000; Alves and Setter, 2004b; Itani et al., 1999). These mechanisms primarily involve water conservation and limited use of carbohydrate such that scarce resources are conserved. Other studies have shown that osmotic adjustment (OA) and accumulation of dehydrin-family Late Embryogenesis Abundant proteins (LEA), a class of desiccation-protectant proteins, is minimally used by cassava, at least in short-term stresses of six to eight days(Alves and Setter, 2000; Alves and Setter, 2004a; Han et al., 2001). Recently, it has been shown that substantial amounts of starch are stored in stems and petioles and are remobilized during a drought stress episode, providing a source of carbohydrate for continued metabolic activity(Duque and Setter, 2005).

Although cassava is considered a drought-tolerant crop, development and final yield are reduced by prolonged dry episodes (Alves, 2002). The decrease in storage root yield depends on the duration and timing of water deficit and the critical period for water stress effect in cassava is thought to be from 1 to 5 months after planting, which encompasses stages of root initiation and tuberization (Deoliveira et al., 1982; Okogbenin and Fregene, 2002). Water deficit during at least 2 months of this period can reduce storage root yield from 32 to 60% (Connor and Cock, 1981; Connor et al., 1981; Porto et al., 1989).

The drought-response traits described above are generally found in cassava; however, diverse genotypes exhibit a wide range of expression for each trait. It is possible that typical trait expression does not confer the optimal combination for crop performance in drought. Furthermore, cassava has been bred for a variety of tropical climates including those with abundant rainfall. Many of the cassava cultivars in these regions have high yield potential, but are prone to yield reduction under drought.

While it is possible to improve drought tolerance by selecting lines for yield in environments that subject the crop to water deficit, many plant breeders and crop physiologists believe more rapid progress can be obtained by pre-breeding research to develop knowledge of the physiological basis of crop performance under drought conditions. A strategy for use of this knowledge involves the breeding of better adapted and higher-yielding cultivars by identifying reliable traits of drought-tolerance such as morphological and/or physiological characters to complement and simplify conventional breeding programs (Bidinger and Witcombe, 1989; Fukai et al., 1999; Ober et al., 2005).

The purpose of the current study was to examine a diverse panel of cassava genotypes and elucidate the extent to which several morpho-physiological traits are associated with drought tolerance during initial storage root developmental stages. Thus the objectives were to (1) evaluate the responses to prolonged water deficit of several traits in contrasting cassava genotypes and (2) determine the interrelationships among morpho-physiological traits affected by water deficit and their association with storage root development. We included 45 contrasting cassava genotypes from the International Center for Tropical Agriculture (CIAT) cassava core germplasm collection. The genotypes were grown in well-watered and water-stressed conditions to elucidate trait responses to water stress and their relationship with storage-root growth.

2.2 Materials and Methods

2.2.1 Plant Material

To determine the effects of artificially induced water stress on several morpho-physiological traits, a field experiment was set up with pot-grown cassava plants, which included 45 contrasting cassava genotypes originating from the International Center for Tropical Agriculture (CIAT) cassava core germplasm collection located in Colombia, South America. Cassava breeders from CIAT and EMBRAPA-CNPMF (Brazil) carefully selected a set of 45 genotypes representative of drought tolerant and susceptible behavior. The selection was based on more than 20 years of yield trials conducted for breeding and crop management research in different agro-ecological areas throughout Colombia and Brazil. The genotypes represent diverse genetic background and climate adaptation, as indicated in Table 2.1.

Table 2.1 List of the 45 cassava genotypes used for well watered and water stressed experiments in this study. The code "*M*" denotes *Manihot*. *CM*, *CG* and *SM* codes identify genotypes derived from CIAT's cassava breeding project. The remaining genotypes are from the germplasm bank collection. The column labeled as "Type" denotes drought tolerant (TOL) or drought susceptible genotypes (SUS).

Genotype	Common name	Origen	Type	Biological status	Selection
MBRA 1133	Veada 1	Brazil	TOL	Landrace	EMBRAPA/CIAT
MBRA 114	Paulo Rosa	Brazil	SUS	Landrace	EMBRAPA/CIAT
MBRA 1142	Guaiana	Brazil	TOL	Landrace	EMBRAPA/CIAT
MBRA 116	Sao Joao	Brazil	TOL	Landrace	EMBRAPA/CIAT
MBRA 1209	CM 425- 7	Colombia	TOL	Improved line	EMBRAPA/CIAT
MBRA 1243	Sapa R-16	Brazil	TOL	Landrace	CIAT
MBRA 134	Rosa (Aipim)	Brazil	TOL	Landrace	EMBRAPA/CIAT
MBRA 1342	MacaxeiraPreta	Brazil	TOL	Landrace	EMBRAPA/CIAT
MBRA 1346	Maragogipe I	Brazil	TOL	Landrace	EMBRAPA/CIAT
MBRA 1394	EnganaLadrao	Brazil	TOL	Landrace	EMBRAPA/CIAT
MBRA 1435	Raimunda	Brazil	TOL	Landrace	CIAT
MBRA 165	Aipim Bravo Preto	Brazil	TOL	Landrace	EMBRAPA/CIAT
MBRA 179	Branca De Sta Cat.	Brazil	TOL	Landrace	EMBRAPA/CIAT
MBRA 200	Do Ceu	Brazil	TOL	Landrace	EMBRAPA/CIAT
MBRA 201	Fio De Ouro	Brazil	SUS	Landrace	EMBRAPA/CIAT
MBRA 209	Manca	Brazil	TOL	Landrace	EMBRAPA/CIAT
MBRA 216	Sacai	Brazil	TOL	Landrace	EMBRAPA/CIAT
MBRA 253	Cachimbo	Brazil	SUS	Landrace	EMBRAPA/CIAT
MBRA 255	EnganaLadrao	Brazil	TOL	Landrace	EMBRAPA/CIAT
MBRA 264	Saracura	Brazil	TOL	Landrace	EMBRAPA/CIAT
MBRA 293	Amansa Burro	Brazil	TOL	Landrace	EMBRAPA/CIAT
MBRA 346	Jaboti	Brazil	SUS	Landrace	EMBRAPA/CIAT
MBRA 534	Pornuncia	Brazil	TOL	Landrace	EMBRAPA/CIAT
MBRA 835	Pretinha V	Brazil	SUS	Landrace	EMBRAPA/CIAT
MBRA 846	Cacau	Brazil	SUS	Landrace	EMBRAPA/CIAT
MBRA 974	Mantiqueira	Brazil	TOL	Landrace	EMBRAPA/CIAT
MBRA 997	Paraguaia	Brazil	SUS	Landrace	EMBRAPA/CIAT
CG 1141-1	ICA-Costeña	Colombia	TOL	Improved line	CIAT
CM 2772-3	N/A	Colombia	SUS	Improved line	CIAT
CM 3306-4	ICA-Negrita	Colombia	TOL	Improved line	CIAT
CM 3306-9	N/A	Colombia	TOL	Improved line	EMBRAPA/CIAT
CM 4919-1	Corpoica Veronica	Colombia	TOL	Improved line	CIAT
CM 507-37	N/A	Colombia	TOL	Improved line	CIAT
MCOL 1468	Mantiqueira	Brazil	SUS	Landrace	EMBRAPA/CIAT
MCOL 1522	Algodoneraamarilla	Colombia	SUS	Landrace	EMBRAPA/CIAT
MCOL 1684	Charay	Colombia	SUS	Landrace	EMBRAPA/CIAT
MCOL 1719	Blanca Mona	Colombia	TOL	Landrace	EMBRAPA/CIAT
MCOL 1734	Negra	Colombia	TOL	Landrace	EMBRAPA/CIAT
MCOL 2066	ChirozaGallinaza	Colombia	SUS	Landrace	EMBRAPA/CIAT
MCOL 2215	Venezolana 1	Colombia	TOL	Landrace	EMBRAPA/CIAT
MPER 183	Eeat 1	Peru	SUS	Landrace	CIAT
SM 1438-2	N/A	Colombia	TOL	Improved line	EMBRAPA/CIAT
MTAI 16	Mkuc 28-77-3	Thailand	TOL	Improved line	EMBRAPA/CIAT
MTAI 8	CMR 246343	Thailand	TOL	Improved line	EMBRAPA/CIAT
MVEN 25	QuerepaAmarga	Venezuela	SUS	Landrace	CIAT

2.2.2 Field Management

Approximately 20 stem cuttings (25-30 cm long) of each genotype were disinfected by thermo-therapy (stakes were immersed in 49 °C water for 49 minutes) and subsequently immersed in a solution of *Trichoderma spp*. (1 kg. DW per 55 gallons of water for 10 minutes). They were sown in 2 kg plastic bags with perforations to allow drainage and containing steam-sterilized mineral soil (5 hours at 90 °C) and coarse sand (2:1), placed in an outside nursery, and received manual irrigation as needed. The soil utilized was an *AquicHapludolf* with the following mean properties at the beginning of the study: pH: 7.65; organic matter: 9.82 g/kg; P (*Bray-II*): 111.37 mg/kg; K: 0.59 cmol/kg; Ca: 6.55 cmol/kg; Mg: 2.51 cmol/kg; Na: 0.15 cmol/kg; CIC: 7.1 cmol/kg; S: 36.82 mg/kg; B: 0.92 mg/kg; Fe: 19.63 mg/kg; Mn: 30.89 mg/kg; Cu: 1.86 mg/kg and Zn: 47.48 mg/kg.

At 60 days after planting (DAP), ten uniformly selected plants of each genotype were transplanted into plastic bags containing ~50 kg (moist weight) of the same soil mixture as described above and watered to field capacity. From these ten plants, five plants were randomly assigned to the well-watered (WW) treatment and the remaining plants assigned to the water-stressed (WS) treatment. The soil bags of all WS plants were covered with a transparent plastic lining sealed at the base of the stem cutting to prevent percolation of naturally occurring rainwater. Perforations in the bags enclosing WW roots allowed drainage of excess water. Afterwards, all plants were taken to the experimental field and randomly placed in a grid (1 m × 1 m). At this stage, referred to as DAY 0, manual irrigation was withheld for WS plants and WW plants were supplied manual irrigation. The experiment consisted of two soil moisture treatments: (i) well-watered control (plants were irrigated every other day until drainage

occurred) and (ii) drought stress (irrigation was withheld and soil was allowed to dry for the duration of the experiment).

2.2.3 Weather Parameters

Weather data for the duration of the experiment were obtained utilizing a HOBO® Temp/RH Data Logger (model H08-032-08, Onset Corporation, U.S.A.) placed within the experimental plot and are presented in Figure 2.1.

The field experiment was conducted during the dry season from May to August 2007 at the CIAT-Palmira experiment station located at 3°29′ N and 76°32'W and 965 meters above sea level. The region where the experiment was set is characterized by a bimodal rainfall pattern (two dry and two wet seasons) each year (Figure 1D).

The driest months are June, July, and August with a monthly precipitation of 55, 28, and 46 mm, respectively. The annual precipitation was 908 mm. The annual mean solar radiation (full-spectrum) for this site is 4.5-5.0 kWh/(m^2 day). The annual potential evapotranspiration for this region is 1343 mm.

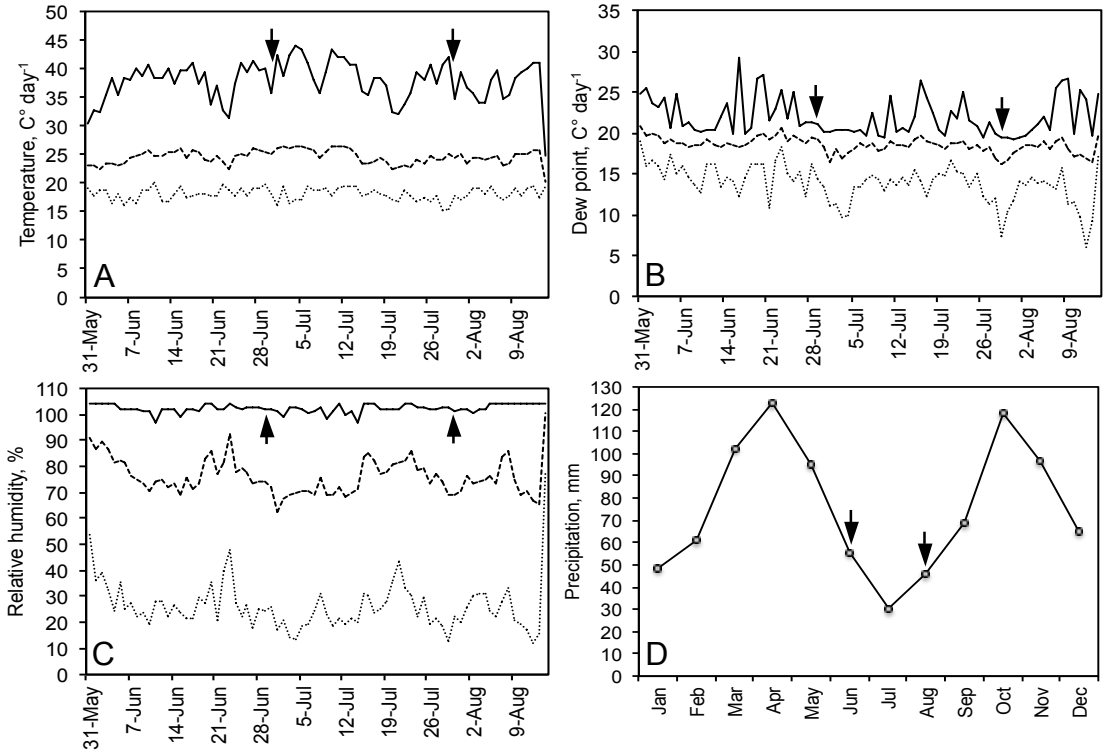


Figure 2.1 Daily temperatures (A), dew point (B), relative humidity (C), and monthly precipitation (D) for the water stress experiment conducted between May 31, 2007 and August 15, 2007. The mean maximum and minimum air temperature during the experimental period ranged between 40 °C and 15 °C. The mean dew point was at 20 °C. Relative humidity oscillated between 100% and 15%. Monthly precipitation represents totals per month. Vertical arrows represent DAY 30 and DAY 60 which are the starting and ending months of the experimental period. Solid lines represent maximum values, dashed lines represent averages, and dotted lines represent minimum values.

2.2.4 Growth Parameters

Two sampling dates were assigned to assess differences between WW and WS treatments within genotypes. The first sampling date was 30 days after the onset of drought (60 DAP), referred as to DAY 30. The second and final sampling date was 60 days after drought onset, referred to as to DAY 60. At DAY 60 all plant material was harvested and yield components evaluated. At each sampling date, the following morpho-physiological traits were recorded (described in detail in later sections): plant height (PH), leaf retention (LR), leaf canopy temperature (T), difference between canopy temperature minus external air temperature (*d*T), leaf chlorophyll greenness (CG; i.e., relative chlorophyll concentration of leaves), volumetric

soil water content (θ;$m^3 \times m^3$), leaf abscisic acid (ABA) and leaf non-structural carbohydrates (NSC) which include: leaf starch (STR), leaf glucose (GLC), leaf sucrose (SUC), and leaf total sugars (TOTSUG). At DAY 60 the following harvest traits were recorded: aboveground biomass fresh weight (AGB_{FW}), storage root fresh and dry weight (SR_{FW}; SR_{DW}), fibrous root fresh and dry weight (FR_{FW}; FR_{DW}), number of storage roots (#SR), and partitioning index (PI),

2.2.4.1 Plant height

Plant height (PH) was measured as the distance from the soil surface at the base of the main stem to the uppermost fully expanded leaf.

2.2.4.2 Leaf Retention

Increased longevity of leaves, or improved leaf retention, has been suggested as a possible means to increase productivity of cassava in water-stressed environments (Lenis et al., 2006). In cassava, leaf abscission advances in a highly predictable pattern starting at the lowest stem node and advancing upward, with retained leaves in the apical section of the stem and branches. The height of the leafless region from the ground to the first (top-most) leaf scar (vacant node) was designated HFS. Leaf retention per genotype was scored on a percentage basis by measuring the total plant height from the soil surface and the length from the first intact leaf-petiole to the uppermost apical meristem on the main stem (height of the stem containing retained leaves, HRL). From these values leaf retention (LR) was calculated from the following expression:

[1] $$LR(\%) = \frac{HRL}{PH} \times 100$$

This procedure was adopted to provide a uniform criterion across a population of genotypes differing in PH.

2.2.4.3 Leaf canopy temperature and differential

Since a major effect of transpiration is leaf cooling, leaf canopy temperature and its reduction relative to ambient air temperature is an indication of the extent to which stomata are open (Brennan et al., 2007). Leaf canopy temperature (T) and the difference in temperature between leaf and external air (*d*T) were measured using a handheld infrared thermometer (model AG-42D, Telatemp Corporation, U.S.A.). The instrument calculates *d*T as ([leaf canopy temperature] – [external air temperature]). The data were taken from the same side of each plant at a 1 m distance from the edge and approximately 50 cm above the canopy at an angle of 30° in reference to the ground. Temperature readings were made between 1100 and 1400 h on cloudless clear sunny days to avoid effects of shading on both sampling dates (Mean solar radiation of 3.83 to 4.14 kWh/m²/day and visibility of 9.7 to 10.3 km). To avoid the effects of soil temperature interfering with canopy temperature the data was taken when the infrared thermometer viewed no soil.

2.2.4.4 Leaf chlorophyll greenness

The SPAD (Soil Plant Analysis Development) or Leaf Chlorophyll meter (Minolta SPAD-502M, Tokyo, Japan) measures leaf chlorophyll, which is an indicator of plant health. The relative chlorophyll concentration is measured in a leaf by quantifying the transmission of red light at 650 nm at which chlorophyll absorbs light, and transmission of infrared light at 940

nm, at which no absorption occurs. With these two values, the instrument calculates a unitless SPAD value between 1 and 100 (Minolta, 1989). The leaf chlorophyll greenness (CG) reading was recorded three times along the midrib of three separate mature fully expanded leaves for each genotype. During measurement, care was taken to ensure that the SPAD meter sensor fully covered the lead lamina and that interference from veins and midribs was avoided. Mean leaf chlorophyll greenness for each genotype was derived from a total of nine measurements per plant.

2.2.4.5 Soil water content

Volumetric soil water content (θ, $m^3 \times m^3$) was measured in the 0-5 cm and 20-25 cm soil layers at DAY 30 and DAY 60 on each plant using a ThetaProbe Soil Moisture Sensor (model ML2x; Delta-T Devices, UK). A set of three-pronged waveguide rods made of stainless steel 20 cm long and 3.0 mm in diameter (Ø), was inserted horizontally in each soil layer and allowed to equilibrate. A total of two measurements per soil layer were taken and averaged.

2.2.4.6 Yield components

At the end of the experiment, aboveground biomass fresh weight (AGB_{FW}), storage root fresh and dry weight (SR_{FW}; SR_{DW}), fibrous root fresh and dry weight (FR_{FW}; FR_{DW}), number of storage roots (#SR) and fresh weight partitioning index (PI) were calculated. All plants were individually harvested at DAY 60. Storage roots were defined as roots of >5 mm diameter (Ø). Partitioning index was measured as the ratio between storage root fresh weight and total biomass expressed in the following equation:

[2] $$PI = \frac{SR_{FW}}{AGB_{FW} + FR_{FW} + SR_{FW}} \times 100$$

2.2.4.7 Leaf non-structural carbohydrates and abscisic acid

For mature and immature leaves, three leaf disks (diameter (Ø) = 0.635 cm each) were sampled from the three most apical fully expanded leaves and three from the three uppermost folded (expanding) leaves to form a composite sample. Leaf disks were sampled at DAY 30 and DAY 60 between 1200 and 1500 hours. Sampled tissue was immediately immersed in 300 µL of cold (0ºC) 80% methanol. All leaf measurements were performed on an area basis.

Leaf sucrose (SUC), glucose (GLC) and starch (STR) were measured on mature fully expanded basal leaves using the peroxidase/glucose oxidase (PGO) method similar to (Ober et al., 1991) and (Setter and Flannigan, 2001). In the PGO method, glucose reacts with O_2 (catalyzed by glucose oxidase) to form gluconic acid and H_2O_2. Catalyzed by peroxidase, the H_2O_2 immediately reacts with *p*-hydroxybenzoic acid and 4-amino-antipyrine to create a bright pink dye complex (Setter and Flannigan, 2001; Trinder, 1969). Crude extracts were used for leaf disk samples analyzed for sucrose and glucose content. Dried sample extracts were re-dissolved in a known volume of 0.01% azide water, and an aliquot was transferred to 96-well plates containing 50 µl autoclaved water. To analyze glucose content, 150 µl of PGO solution (peroxidase and glucose oxidase enzymes in buffer solution containing 100 mM KH_2PO_4-NaOH (pH 7.0), 10 mMpara-hydroxybenzoic acid, 0.001 mM 4-aminoantipyrine, 0.1% (w/w) bovine serum albumin, and 0.01% sodium azide) were added to each well containing leaf and stem samples. After full color development at room temperature, the plates were read on a Packard SpectraCount model 750 photometer (490 nm wavelength setting).

To quantify total sugars (including sucrose) content, an invertase solution (292 U/mg, 10mg/mL H_20) was added to the samples, and reaction was allowed to run until full color development of sucrose standards before reading on the photometer (490 nm). Standards made from dilutions of glucose (0 to 32 µg/well) and sucrose (25 µg/well) solutions were used to calibrate the assay.

After all free sugars were extracted; starch content was also determined in the insoluble fraction. After samples were dried overnight, each sample was rediluted in 200 µL azide water, covered, and incubated at 80°C for two hours to gelatinize starch. Samples were cooled and 200 µL enzyme solution (250 mM acetate buffer at pH 4.5, 74 U/mg amyloglucosidase, 20 U/mg α-amylase, 0.1% w/v sodium azide, and 0.1% BSA) were added to hydrolyze starch into glucose. The reaction was incubated for two days on a rotary shaker at 37°C. Samples were then stored at 5°C. The PGO method was then used to determine the amount of glucose cleaved from starch.

Prior to hormone analysis, leaf tissue was first separated into fractions based on hydrophobicity using reverse phase C_{18} chromatography, modified from Setter et al. (2001). Supelco columns (DSC-18 SPE-96) with 25 mg of C_{18} packing material were used in a 96-well vacuum apparatus. Columns were washed with 95% ethanol and 30% methanol prior to use. Extracts from samples stored in 80% methanol were transferred to a 96-well plate, dried in a forced-air incubator at 45°C, then redissolved in 100 µL 30% methanol and 1% v/v glacial acetic acid with 20 µL 0.04% bromecresol green added as a chromatograph tracer. Samples were loaded onto the columns with 120 µL 30% methanol, and pulled through by vacuum. Columns were then washed with 200 µL 30% methanol to remove any remaining hydrophilic compounds.

Abscisic acid was eluted from the columns using 200 µL 65% methanol with 1% acetic acid. Then columns were subjected to 200 µL 95% ethanol to remove any lingering compounds.

NH_4OH was added to neutralize the acetic acid. Plates were read on a spectrophotometer (Packard SpectraCount model 750) using a 590 nm wavelength to measure bromecresol green.

Abscisic acid (ABA) levels were determined using an enzyme-linked immunosorbant assay (ELISA) as described in (Setter and Flannigan, 2001). The 65% methanol fractions from reverse phase C_{18} chromatography were used for ABA quantification. After drying, samples were redissolved in 100 µL azide water (0.01%). Next, 96-well plates were coated overnight with a BSA conjugate solution (ABA-BSA for abscisic acid) containing 1.4 µg BSA conjugates per plate and 50mM $NaHCO_3$ at a pH of 9.6. Plates were then washed four times with a TBS (10mM tris-hydroxymethylaminomethane-HCl, pH of 7.5, 1 mM $MgCl_2$, 100mM NaCl) and 0.1% Tween-20 solution (TBST). Dried samples were redissolved in 200 µL of water (containing 0.01% azide) and 20 µL was dispensed into 90 µL of MBSA (50mM MOPS-NaOH, pH 7.5, 1 mM $MgCl_2$, 100 mM NaCl, 0.1% bovine serum albumin) and 100 µL of primary antibody solution (100 µL MBSA containing 1 µg of anti-ABA; clone #15-I-C5, Mertens, Deus et al., 1983; currently available from Agdia Inc., Elkhart, IN) (Setter et al., 2001). A calibration curve was generated using a serial dilution of ABA standards ranging from 0.002 to 5 pmol/well. After incubating overnight at 5°C, the plates were again washed four times with a TBST solution. Secondary antibody solution (200 µL containing 10 nL of anti-mouse IgG-alkaline phosphatase (reporter enzyme) conjugate in MBSA) was added to each well. Reaction was run overnight at 5°C. After washing four times with a TBST solution, 200 µL PNPP (0.2 mg *p*-nitrophenyl phosphate in 0.9M diethanolamine and 3 mM $MgCl_2$ at pH 9.8) substrate solution was added and the reaction incubated for 60 min at room temperature before reading absorbance at 405 nm with spectrophotometer (Packard, model: SpectraCount).

2.2.5 Statistical Analysis

All data were subjected to statistical analysis, using a JMP 9.0 statistical package (SAS Institute, Inc. U.S.A.). All results presented are means ± sem. Least significance differences (LSD) between resulting means were estimated by Tukey's HSD test for multiple comparisons ($p< 0.05$). For each treatment, phenotypic associations between all traits measured were estimated as *Pearson* product-moment *correlation* coefficient to determine trends in the data.

Genotypic correlations between two traits, 1 and 2, within the same environment were calculated as:

[3] $$r_{G12} = \frac{Cov_{12}}{\sqrt{\sigma^2_{G1} \times \sigma^2_{G2}}}$$

(Bernardo, 2002; Bernier et al., 2007; Kumar et al., 2007), where r_{G12}, Cov_{12}, $\sigma^2_{G1}$and$\sigma^2_{G2}$are the genetic correlation coefficient between traits 1 and 2 within the same environment, genetic covariance of traits 1 and 2, and the genotypic variances of traits 1 and 2, respectively. This estimation method assumes that the covariance between genotype means is entirely caused by correlation of genotypic effects and that there are no environmental effects apart from the uncontrolled residual effects between replicate plants. Genetic correlations were reported only when the phenotypic correlation between the two traits was significant ($P \leq 0.05$). In a recent study by (Kumar et al., 2007), resulting covariance and variance component estimates had large sampling errors, thus estimates of r_G were imprecise. Hence, these results should be considered as rough guides to the degree of genetic association between traits. Genetic correlations estimated from linear functions of covariances tend to "over-correct" phenotypic correlations when H^2 for one or both of the traits involved is very low, resulting in estimates substantially

greater than 1 or less than -1 (Kumar et al., 2007). As a result, genetic correlations for such traits were not reported for this experiment.

2.3 Results

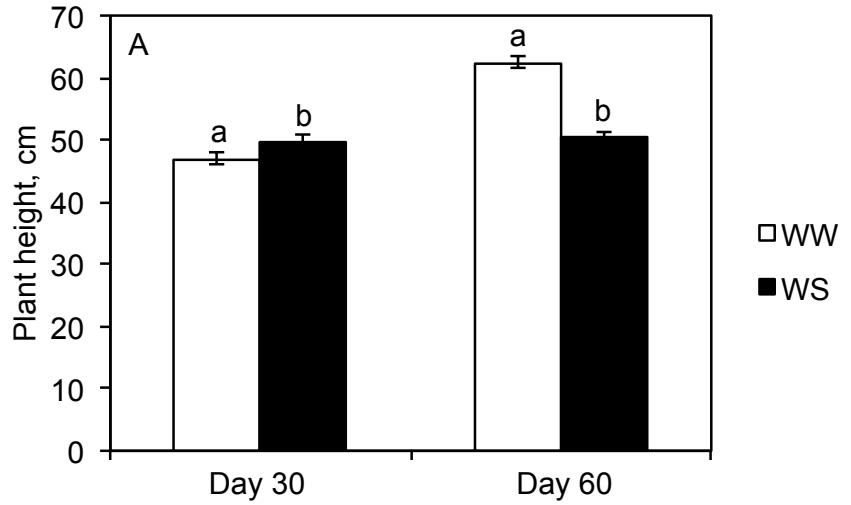
Genotype, treatment (water stressed versus well-watered), and genotype × treatment interaction effects were highly significant ($p \leq 0.01$) for most parameters evaluated (data not shown; Appendix Table A1). At DAY 30, WS plants were slightly taller than WW plants (5%), indicating that the stress was at an early stage at that sampling date. However, from DAY 30 to DAY 60, WW plants increased plant height (PH) by 25% whereas WS plant height increased only 2% (Figure 2.2 A).

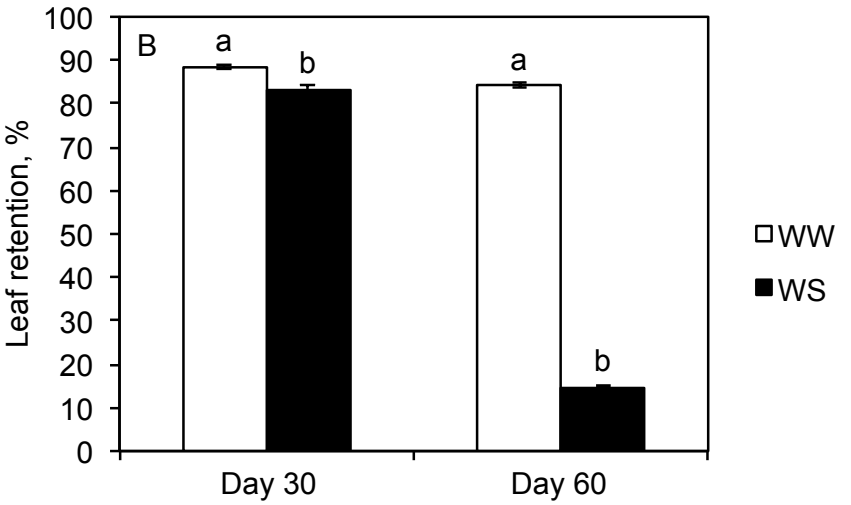
Leaf longevity was evaluated by leaf chlorophyll greenness and leaf retention (as opposed to abscission). Abscission of lower leaves resulted in a large decline in leaf retention in the WS plants; between DAY 30 and DAY 60 leaf retention in WS plants decreased from 83 to 15%, while leaves in WW plants retained their leaves (< 4% change; Figure 2.2. B). In contrast, leaf chlorophyll greenness (CG) in the retained leaves was relatively unaffected or increased slightly in response to stress. At DAY 30, measured leaves in the WS treatment had about a 12% higher greenness score than WW leaves, while at DAY 60 both treatments had decreased greenness slightly and were not significantly different from each other (Figure 2.2. E).

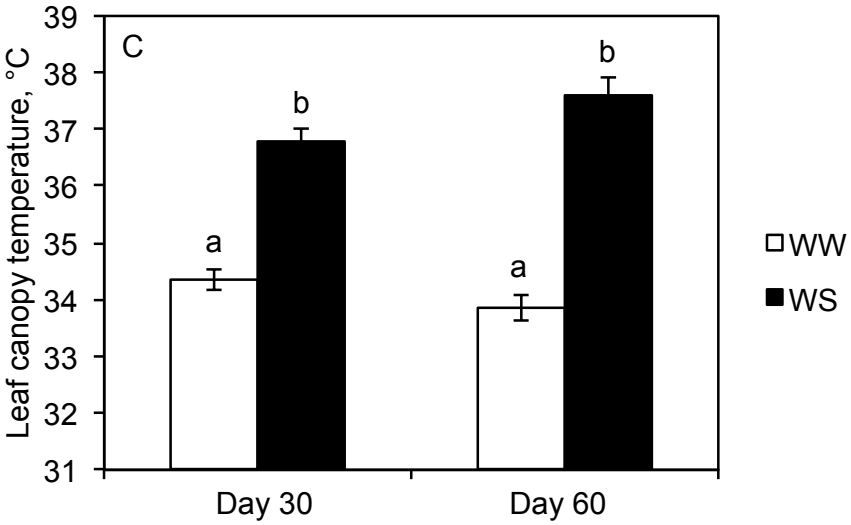
Leaf canopy temperature (T) and leaf-air temperature difference (dT) were examined to determine effects of water stress on foliar temperature due to decline in evaporative cooling with stomatal closure. Consistent with stomatal closure, T values in WS plants were approximately double those in WW plants both at DAY 30 and at DAY 60 (Figure 2.2. C). In addition, dT

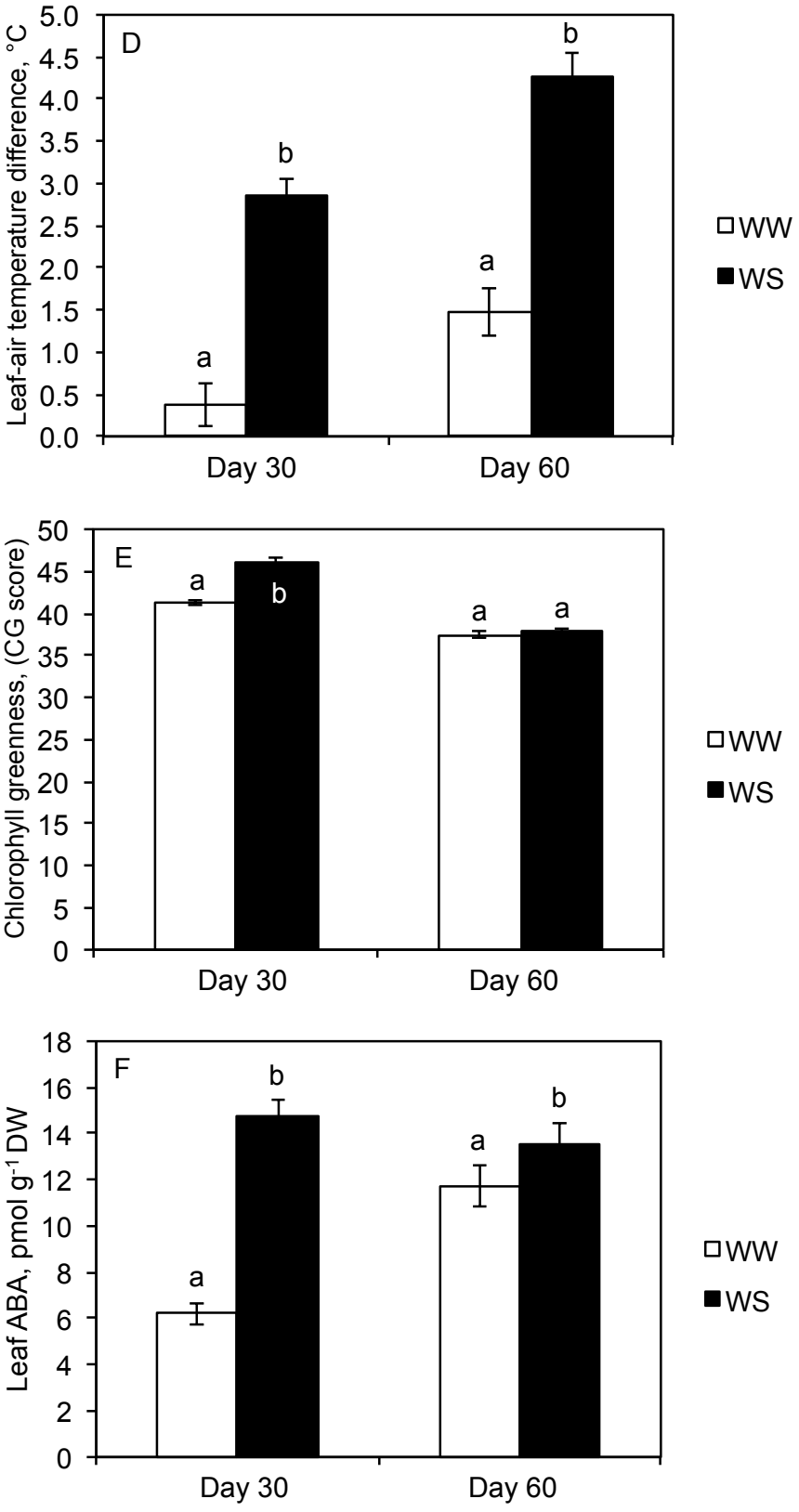
values were 5-fold higher in WS than WW at DAY 30 and 2-fold higher at DAY 60 in WS plants (Figure 2.2. D). Consistent with this observation, WS plants had higher leaf abscisic acid (ABA) concentration (~15 pmol/mg DW) on DAY 30 when compared to WW plants (~6 pmol/mg DW) (Figure 2.2. F). In WS plants, leaf-air temperature difference increased further in the interval from DAY 30 to DAY 60. In this interval, *d*T also increased in the well-watered plants from 0.4 to 1.5 °C, suggesting that their stomata closed somewhat (Figure 2.2. D). Consistent with *d*T indicating stomatal closure, at DAY 60 the well-watered plants accumulated leaf ABA such that both groups had approximately equal leaf ABA concentrations (12-14 pmol/mg DW; Figure 2.2. F).

Figure 2.2 Plant height (A), leaf retention (B), leaf canopy temperature (C), leaf-air temperature difference (D), chlorophyll greenness (E) and leaf ABA (F) of cassava under well-watered (WW) and water-stressed (WS) conditions and measured at DAY 30 and DAY 60. Vertical bars are standard errors, n = 5 per treatment-genotype combination. For each pair of WW and WS treatments (each sampling date), bars with different letters indicate a significant difference ($p \leq 0.05$) between them.


A
Plant height, cm
70
60
50
40
30
20
10
0
a
b
a
b
Day 30
Day 60
WW
WS


B
Leaf retention, %
100
90
80
70
60
50
40
30
20
10
0
a
b
a
b
Day 30
Day 60
WW
WS


C
Leaf canopy temperature, °C
39
38
37
36
35
34
33
32
31
a
b
a
b
Day 30
Day 60
WW
WS


D
Leaf-air temperature difference, °C
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
a
b
a
b
Day 30
Day 60
WW
WS
E
Chlorophyll greenness, (CG score)
50
45
40
35
30
25
20
15
10
5
0
a
b
a
a
Day 30
Day 60
WW
WS
F
Leaf ABA, pmol g-1 DW
18
16
14
12
10
8
6
4
2
0
a
b
a
b
Day 30
Day 60
WW
WS

Within each treatment and sampling date, there was about the same soil water content at 5 and 25 cm depths, indicating that water depletion was fairly uniform throughout the soil profile. At both DAY 30 and DAY 60, WW pots had significantly (P≤0.05) higher soil water content than their WS counterparts. However, despite the intent to keep WW plants at high water status with daily irrigation, some depletion of soil water occurred by DAY 60 in the WW pots. This was probably due to WW plants developing large leaf canopies by DAY 60, leading to a large amount of daily transpiration relative to pot size and soil water holding capacity. In the WS plants, water content at DAY 60 was only 0.02% (0-5 cm) and 0.03% (20-25 cm). Water contents were quite similar between genotypes, probably because each genotype depleted water until stomatal closure, which would drastically slow further water depletion and represent a lower limit for soil water content.

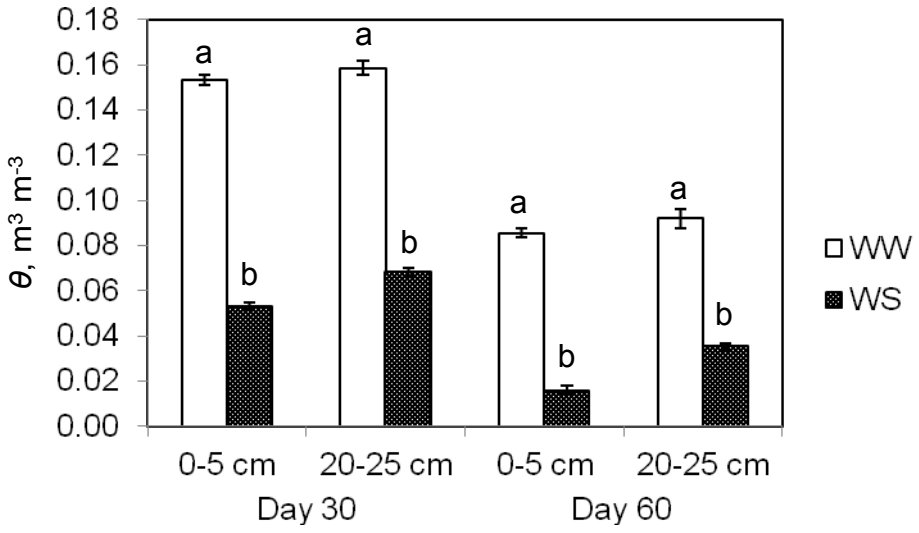


Figure 2.3 Volumetric soil water content (θ) of well-watered (WW) and water-stressed (WS) pots measured at 0-5 cm and 20-25 cm depth at 30 and 60 days. Vertical bars are standard errors, n = 5 per treatment-genotype combination. For each pair of WW and WS treatments (each sampling date), bars with different letters indicate a significant difference ($p \leq 0.05$) between them.

At DAY 60, WS and WW plants were harvested and their yield components analyzed (Figure 2.4). Water-stressed plants' aboveground biomass fresh weight (AGB_{FW}) was ≤ 65% of comparable well-watered plants (Figure 2.4. A). Furthermore, storage and fibrous root dry weights (SR_{DW} and FR_{DW}) were markedly decreased due to water stress. Comparable treatment effects were documented on fresh weights of these organs (data not shown). Water stress decreased SR_{DW} and FR_{DW} 88% and 46%, respectively (Figure 2.4. B and C). Number of storage roots (#SR) was ~50% lower and partitioning index (PI) in WS plants were ~33% of the WW plants, (Figure 2.4. D and E).

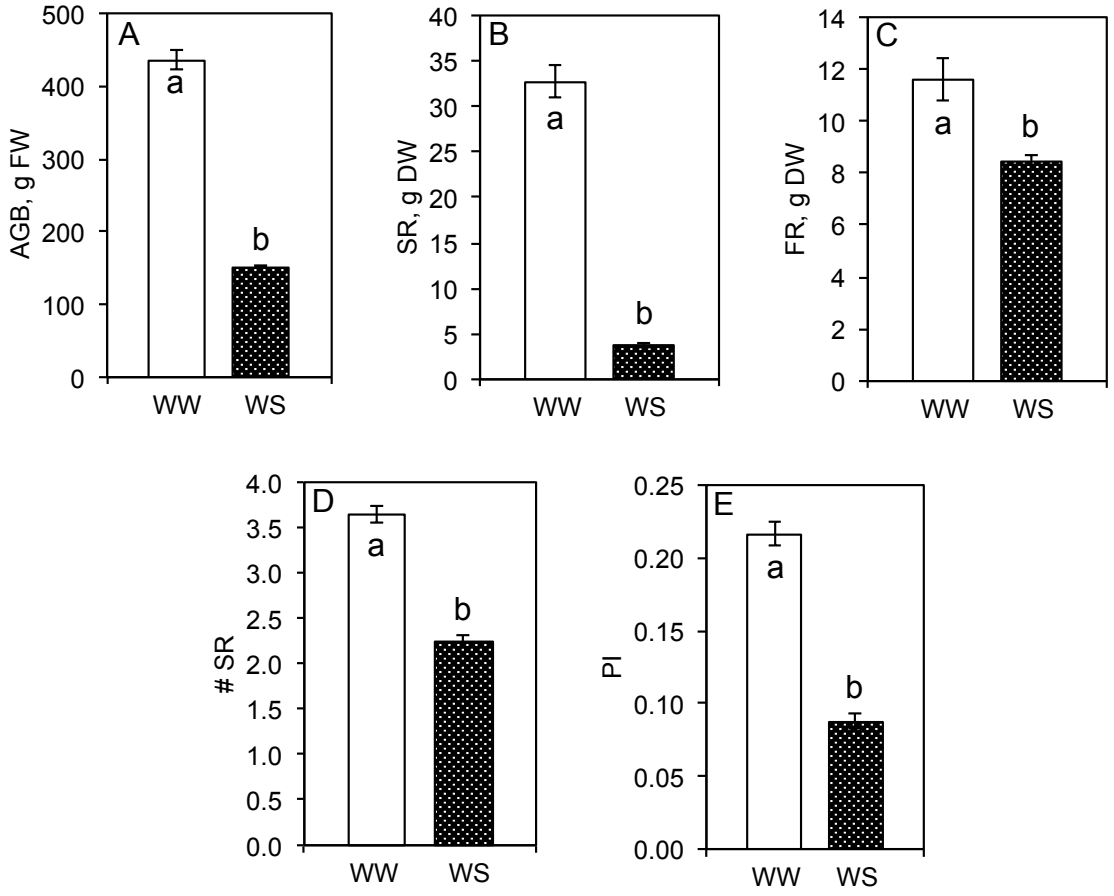
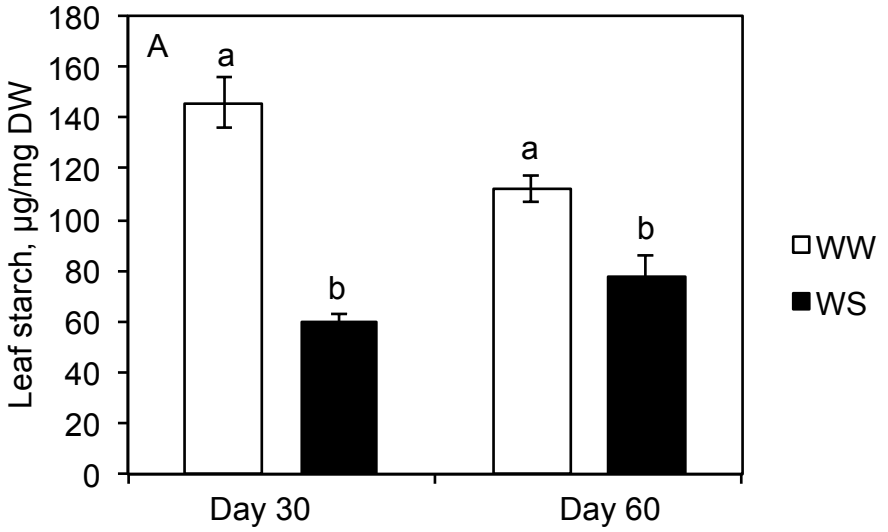
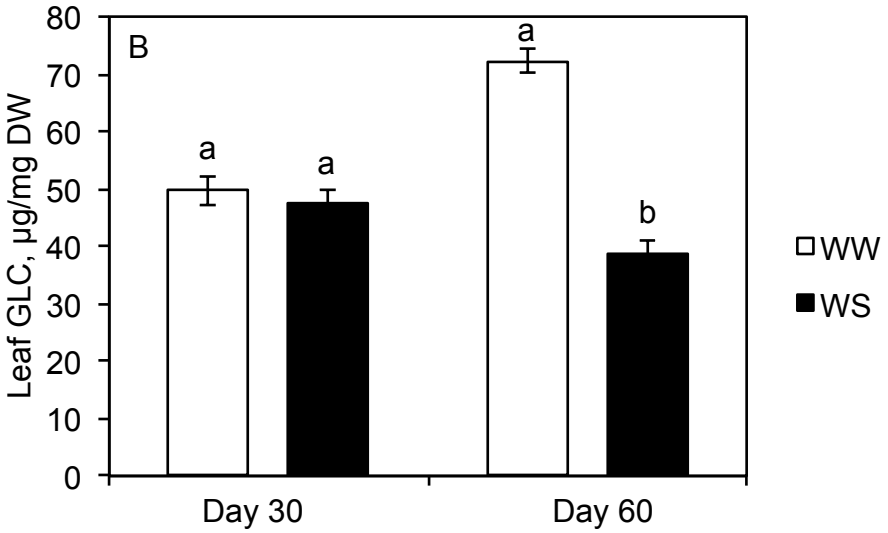


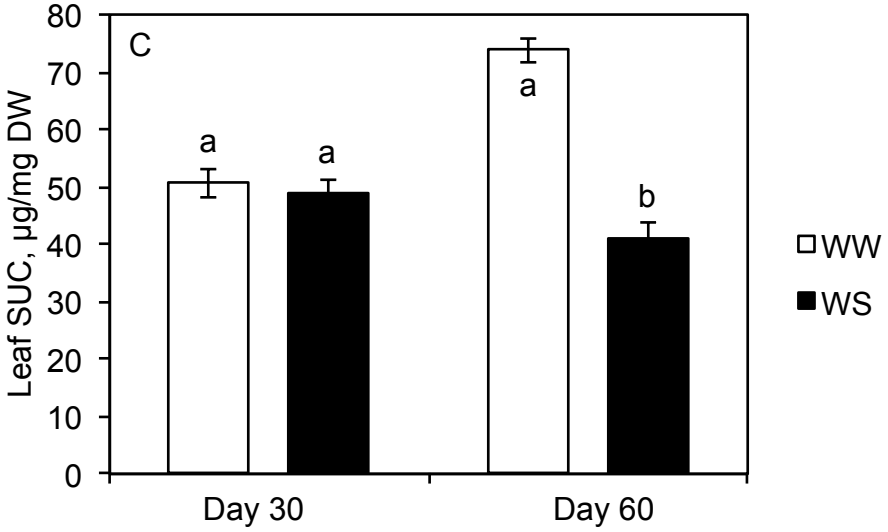
Figure 2.4. Aboveground biomass fresh weight (A), storage root dry weight (B), fibrous root dry weight (C), number of storage roots (D) and partitioning index (E) of cassava under well-watered (WW) and water-stressed (WS) conditions measured at harvest (DAY 60). *n*= 5 per treatment-genotype combination.

Water stress also affected leaf non-structural carbohydrates (NSC) (Figure 2.5). NSC levels could provide an indication of net photosynthetic carbon gain, while sugar levels may indicate the tendency for osmolyte accumulation in response to WS. In WW treatments, there was a general decline in leaf starch from DAY 30 to DAY 60 (>27%). However, in WS treatments, though leaf starch was lower when compared to WW treatments in both sampling days, WS leaf starch increased from DAY 30 to DAY 60 (>25%). Leaf glucose and sucrose (GLC and SUC) also declined from DAY 30 to DAY 60 in the WS leaves. At DAY 30, leaf GLC and SUC presented similar values in both WW and WS treatments. However at DAY 60, WW leaf GLC and SUC was approximately two fold when compared to its WS counterparts.

Figure 2.5 Leaf non-structural carbohydrates of cassava under well-watered (WW) and water-stressed (WS) conditions measured at DAY 30 and DAY 60. Vertical bars are standard errors, n = 5 per treatment-genotype combination. STR = starch, GLC = glucose, SUC = sucrose. For each pair of WW and WS treatments (each sampling date), bars with different letters indicate a significant difference ($p \leq 0.05$) between them.


A
Leaf starch, µg/mg DW
180
160
140
120
100
80
60
40
20
0
a
b
a
b
Day 30
Day 60
WW
WS


B
Leaf GLC, µg/mg DW
80
70
60
50
40
30
20
10
0
a
a
a
b
Day 30
Day 60
WW
WS


C
Leaf SUC, µg/mg DW
80
70
60
50
40
30
20
10
0
a
a
a
b
Day 30
Day 60
WW
WS

While the effects of water stress and well-watered conditions were averaged across all genotypes in the results shown above, phenotypic correlations were used to assess the associations between traits for the range of values measured in the genotypes (data not shown, Appendix Table A2). As described in Appendix Table A2, the diagonal matrix in the upper right of the table shows correlation coefficients for WS plants and the diagonal matrix in the lower left shows statistical significance values. For WW plants (Appendix Table A2 continued), the diagonal matrix in the lower left of the table shows correlation coefficients for WW plants and the diagonal matrix in the upper right shows statistical significance values.

Regarding yield components, storage root fresh weight (SR_{FW}) in WW plants was correlated with number of storage roots (#SR) (0.57; $p \leq 0.01$***), and SR_{FW} in WS was not correlated with #SR (0.25; ns). Storage root fresh weight in both WW and WS was not correlated with above ground biomass ($r < -0.07$ and $r < -0.12$, respectively); however, they had strong correlations with storage root partitioning index (*PI*) (0.81*** and 0.93*** in WW and WS, respectively). This was related to the altered partitioning of biomass between alternative sink organs. In WS plants, partitioning index was negatively correlated with plant height at DAY 30 ($PH_{30,}$ -0.45***) and DAY 60 ($PH_{60,}$ -0.49***), as well as aboveground biomass as a whole (-0.41**). Similarly, in WW plants, partitioning index was negatively correlated with plant height at DAY 30 ($PH_{30,}$ -0.39**) and DAY 60 ($PH_{60,}$ -0.42**), with fibrous root DW (-0.45***), and with aboveground biomass as a whole (-0.58***).

In WS and WW treatments, leaf retentions at both DAY 30 and DAY 60 (LR_{30} and LR_{60}) were not significantly correlated with storage root fresh weight. The height of the stem region that retained leaves at DAY 30 and DAY 60 (HRL_{30} and HRL_{60}) was also not significantly correlated with storage root fresh weight. HRL_{60} was positively correlated with LR_{60} (0.77***).

However, even though HRL is a more direct measure of retained leaf area than LR_{60}, HRL_{60} was poorly correlated with all measures of growth, including plant height, above ground biomass, and storage root fresh weight. LR_{30} and LR_{60} are measures of the percentage of height in which nodes have retained leaves, and as such they could also be affected by plant height and the size of the abscised region. Consistent with this possibility, in WS, LR_{60} was negatively correlated with PH_{60} (-0.40**) and with height of the leafless region from the ground to the first (top-most) leaf scar (vacant node) (HFS_{60}; -0.61***). Furthermore, in WS, PH_{60} was strongly correlated with HFS_{60} (-0.97***) and not correlated with HRL_{60} (-0.23, ns). This indicates that genotypes in WS varied the size of their leaf scar regions (HFS) in proportion to PH, but were relatively similar in the size of their retained-leaf regions (HRL).

Even though the average across all genotypes showed that water stress only slightly decreased leaf retention at DAY 30 (Fig. 2.2. B), when differences were compared among genotypes, leaf retention at Day 30 (LR_{30}) was not correlated with leaf ABA levels at DAY 30 (-0.29, ns) and with soil water content in the 0 to 5 cm zone ($\theta_{0\text{-}5(30)}$, -0.42***). This suggests that genotypes retain leaves in the early phase (up to DAY 30) accumulate less ABA and deplete soil water more rapidly. However, LR_{60}, which was severely decreased by water stress, was not correlated with either ABA or soil water content. At DAY 60, soil water was largely depleted and ABA was substantially increased in all the genotypes. Chlorophyll content of retained leaves, which was assessed by CG, was not correlated with the percent leaf retention on a plant.

Several measures of leaf carbohydrate levels in the WS treatment were correlated with plant water status. Leaf starch levels at DAY 30 and DAY 60 were negatively correlated with leaf ABA at DAY 60 (-0.40** and -0.34*, respectively). This is consistent with the possibility that genotypes, which accumulated more ABA, had more closed stomata and fixed less CO_2.

Leaf sucrose at DAY 30 was positively correlated with soil water content at DAY 30 ($\theta_{0\text{-}5(30)}$, r = 0.43**) and leaf glucose at DAY 60 was correlated with soil water content at DAY 60 in both 0-5 and 20-25 cm depths (0.31* and 0.46***, respectively). Associations of leaf carbohydrate with ABA were also observed in well-watered conditions; however, the functional relationships were complex. At DAY 30, sucrose was positively correlated with ABA at DAY 30 (0.39**) and at DAY 60 (0.44**).

In the WS treatment, ABA at DAY 30 was positively correlated with soil water content at DAY 30 (0.39**, 0-5 cm depth) but not at DAY 60 (0.12; ns, 0-5 cm depth), consistent with a model whereby genotypes with high ABA conserve water. Negative correlations (i.e. ABA and leaf-air temperature difference at DAY 30 and DAY 60) are contrary to a model whereby genotypes with high ABA have more stomatal closure and associated canopy warming. Another factor is that canopy temperature was measured somewhat earlier in the day than when leaf tissue was sampled for ABA determination. If certain genotypes have high ABA in the afternoon when elevated temperature creates high leaf-air vapor pressure deficit, they might conserve soil moisture sufficiently to allow them to have more open stomata in earlier times of the day.

Genotypic tendency to accumulate ABA was quite consistent across WS and WW treatments (data not shown). ABA levels at DAY 30 in the WS treatment were correlated with WW ABA at DAY 30 (0.78***) and at DAY 60 (0.53***), and WS ABA at DAY 60 was correlated with WW ABA at DAY 60 (0.44**).

Genotypic correlations between all traits measured, yield, and yield components provide information on expected responses in yield and yield components from selection for secondary traits. Genotypic correlations under stress and well-watered environments were assessed at DAY

30 and at DAY 60, however only a subset of trait genotypic correlations is given for both sampling days. In addition, both phenotypic and genotypic correlations provided comparable information and are presented in Appendix Tables A2 and A3.

A high and positive correlation (0.94** and 0.84**) was observed between storage root fresh weight (SR_{FW}) and partitioning index (PI) under stress and in well-watered conditions, respectively. Plant height at DAY 60 (PH_{60}) was significantly but moderately negatively correlated with SR_{FW} under stress (-0.35*; $p \leq 0.05$). Interestingly, SR_{FW} in well-watered conditions was negatively correlated with fibrous root fresh weight (FR_{FW}) (-0.33*; $p \leq 0.05$) and highly positively correlated with number of storage roots (#SR) (0.60**; $p \leq 0.01$). All other trait combinations with storage root fresh weight (SR_{FW}) under stress and well-watered conditions resulted in no correlations. In general, nonstructural carbohydrates (NSC) and abscisic acid (ABA) were significantly and negatively correlated with SR_{FW} under stress and well-watered conditions. On the other hand, leaf starch at DAY 60 (STR_{60}) was moderately positively correlated with SR_{FW} (0.38**; $p \leq 0.01$) under stress. Regarding other trait combinations, leaf retention at DAY 60 (LR_{60}) presented positive correlations with leaf chlorophyll greenness (CG_{60}), leaf starch (STR_{60}) and partitioning index (PI) at DAY 60 (0.41**; 0.38**; $p \leq 0.01$; 0.32*; $p \leq 0.05$, respectively) and a negative correlation with above ground biomass fresh weight (ABG_{FW}) (-0.40**; $p \leq 0.01$) under water stress conditions. Conversely, under well-watered conditions these same trait combinations were non-significant, except for leaf starch (STR_{60}), which was highly negatively correlated (-0.87**; $p \leq 0.01$). Plant height at DAY 60 (PH_{60}) under water stress presented negative correlations with leaf glucose (GLC_{60}), leaf total sugars ($TOTSUG_{60}$), and partitioning index (PI).

In well-watered conditions, plant height (PH_{60}) was positively correlated with aboveground biomass fresh weight (AGB_{FW}) and fibrous root fresh weight (FR_{FW}) but negatively correlated with number of storage roots (#SR) and partitioning index (PI). In addition, PH_{60} was negatively correlated with all leaf non-structural carbohydrates (NSC) and abscisic acid (ABA) traits. Leaf canopy temperature (T_{60}) at DAY 60 under water stress was significantly correlated with leaf starch (STR_{60}) and abscisic acid (ABA_{60}) (-0.82** and -0.77**; $p \leq 0.01$, respectively). Also, ABA_{60} was negatively correlated with T_{60} under well-watered conditions (-0.57**; $p \leq 0.01$). Leaf chlorophyll greenness at DAY 60 (CG_{60}) was negatively correlated with leaf glucose (GLC_{60}) and sucrose (SUC_{60}) under stress (-0.57** and -0.59**; $p \leq 0.01$, respectively). Under well-watered conditions, CG_{60} was positively correlated with aboveground biomass fresh weight (AGB_{FW}) but negatively correlated with all leaf non-structural carbohydrates (NSC) and abscisic acid (ABA) traits. Regarding volumetric soil water content at 0-5 cm depth ($\theta_{0\text{-}5}$), a moderately negative correlation resulted when compared to leaf canopy temperature (T) (-0.42**; $p \leq 0.01$) under stress at DAY 60. Similar results were assessed at $\theta_{20\text{-}25}$, (-0.50**; $p \leq 0.01$). Under well-watered conditions at DAY 60, volumetric soil water content at both soil depths gave a moderately negative correlation when compared to leaf chlorophyll greenness (CG_{60}) (-0.42** and -0.45**; $p \leq 0.01$, respectively). In addition, above ground biomass fresh weight (ABG_{FW}) and fibrous root fresh weight (FR_{FW}) were negatively correlated with volumetric soil water content at both soil depths under well-watered conditions at DAY 60.

2.4 Discussion

To make progress in developing improved cassava cultivars, it is valuable to know which traits and mechanisms underlie its tolerance to prolonged drought in its region of adaptation in the tropics. In the current study we have reported the responses of several morpho-physiological traits in association with drought tolerance during the initial storage root developmental phase. While previous studies on traits such as stomatal conductance, soil water depletion, ABA accumulation, and leaf non-structural carbohydrate accumulation have been conducted on limited sets of one to six genotypes and in studies taking one physiological attribute at a time, the current study examines a broader panel of 45 diverse genotypes and a larger number of traits, thereby providing a better assessment of the association of these traits to drought performance. The current study also included traits such as leaf retention and partitioning index at early storage root initiation, which previous surveys of genetic populations have indicated may have potential contributions to drought performance.

Accordingly, the data will be presented in two general ways: 1) the main effects of water stress treatment on each trait, averaged across all genotypes, to provide a general characterization of water stress response in cassava, and 2) phenotypic correlations of traits. Phenotypic correlations were used to examine relationships between traits over the range of variation provided by the 45 genotypes.

While it is acknowledged that genetic and environmental factors combine to result in a phenotypic correlation, in the current study, field experiments were performed in a single crop year on plants grown in pots with a defined volume of uniform soil, and other environmental factors such as water supply and pests were controlled. Thus, the phenotypic correlations

reported here are likely to represent largely genotypic components of correlation, while environmental and G×E components are minimized.

These data are potentially useful in identifying traits for which there is genetic variation that correlates with yield and stress tolerance. Such correlated traits merit consideration for future effort to phenotype cassava germplasm. The phenotypic correlations can also identify relationships between traits that help us better understand the underlying mechanisms of water stress response. While emphasis in crop breeding is on breeding for improved yield, there has been interest in using an advanced understanding of underlying physiology to identify secondary traits for genetic selection. To be useful, secondary traits should be correlated with yield and other important essential attributes, and be heritable and variable in the populations used for breeding. In several crops there has been encouraging progress in the use of physiological or secondary traits as indirect selection criteria for drought tolerance, such as succulence index and wilting score in sugar beet (Ober et al., 2005); spectral reflectance indices in wheat (Babar et al., 2006); lower WUE, higher leaf N, and larger leaf size in sunflower (*Helianthus annuus* L.) (Donovan et al., 2007); and early vigor in rice (Zhao et al., 2006).

With respect to plant height, the growth pattern from DAY 30 to DAY 60 shows that at DAY 30 the stress was in an early stage of development, while subsequent growth and dry matter accumulation was significantly impaired between Day 30 and Day 60 of water stress. In the DAY 30 to DAY 60 interval, volumetric soil water content decreased about 2-fold. In similar studies, it has been reported that decreased growth and development of shoots in cassava is susceptible to rather mild soil water content variations (Calatayud et al., 2000; Duque and Setter, 2005; Pardales and Esquibel, 1996). Expansion growth of leaves and shoots in plants is known to be among the earliest processes to respond to incipient water deficit (Hsiao and Xu, 2000). In

cassava, this sensitivity of leaf and stem growth to mild stress acts in concert with a large set of responses including stomatal closure and leaf abscission, which conserve water and limit respiratory and carbohydrate demand during stress (El-Sharkawy, 2006; Setter and Fregene, 2007). In the current study, phenotypic correlations for the panel of diverse genotypes in the water stress treatment showed that plant height was negatively associated with storage root biomass in water stress, consistent with the possible advantage of limiting the amount of photosynthate used for shoots such that storage root growth is favored.

Numerous reports indicate that better leaf retention could be a key factor for achieving high yields in cassava (El-Sharkawy, 2003; Lenis et al., 2006). Nevertheless, cassava has been previously reported to respond to water stress with substantial leaf senescence and abscission (Duque and Setter, 2005; El-Sharkawy et al., 1992a; Porto et al., 1989). In our study, leaf retention decreased rapidly from DAY 30 to DAY 60 under stress while plants under well-watered conditions largely retained their leaves. In the current study, leaf retention was poorly correlated with storage root fresh weight. This may have been because all genotypes tended to retain at least a small number of leaves near the shoot apex at the end of the experiment, which probably provided sufficient photosynthetic productivity. Consistent with this possibility, the percentage of stem length with retained leaves (RL) was negatively correlated with plant height in water stress. Nevertheless, the height of the portion of stems with retained leaves (HRL) was poorly correlated with all measures of growth, including aboveground biomass and storage root growth. These data indicate that care is needed in interpretation of leaf retention data as the relationship of RL to drought tolerance could be complicated by the method of expressing the data. Also, leaf retention can have countervailing effects on plant performance during drought by favorably preserving photosynthetic potential while having detrimental effects by increasing

carbon respiratory consumption and transpiration. Consistent with this possibility, there was a negative correlation between RL and soil water content at DAY 30.

The current results are in contrast with those of Lenis et al. (2006), in which positive correlations were found between leaf retention and overall fresh root yield (0.43). Possible confounding factors in a comparison with the current results include differences in the severity, type, and duration of the water stress episode in these investigations. In addition, our experiment was harvested at about six months after planting while Lenis et al. (2006) harvested at maturity (12 months after planting). In spite of this, leaf retention/abscission/longevity are traits easily measured in the field and can give valuable information regarding drought responses.

This finding that leaf abscission tends to proceed until a limited height of leaves remains near the apex is in agreement with Duque and Setter (2005). In that study it was also observed that several cassava genotypes produced new leaves even in the face of stress during stress imposition (albeit reduced in size). Studies done by Conner and Cock (1981), Alves and Setter (2000), and Duque and Setter (2005) demonstrated that cassava genotypes do not abscise immediately after entering a drought episode, but abscission progresses gradually and overall leaf area is also a function of the rate of new leaf production.

Leaf chlorophyll greenness index can be estimated non-destructively in single leaves with a hand-held meter such as the Minolta SPAD meter. Many studies have been performed utilizing this technique in reference to chlorophyll content, nitrogen status, and water stress (Fanizza et al., 1991a; Fanizza et al., 1991b; Haripriya Anand and Byju, 2008; Naderikharaji et al., 2008). Our results indicated that after DAY 60, leaf chlorophyll greenness readings on cassava plants under stress were similar to those in well-watered conditions, probably because the uppermost leaves did not senesce and retained their function or represented new leaf production as noted

above. Conversely, Naderikharaji et al. (2008) working with rapeseed (*Brassica napus*) and Fanizza et al. (1991a) with table grapes (*Vitisvinifera*) found that water stress decreased SPAD readings by harvest time or after two weeks after the onset of drought, respectively. In contrast to the current study with drought stress, HaripriyaAnand and Byju(2008) determined in studies of nitrogen nutrition of cassava that the relationship between tuber yield and SPAD, leaf color chart score (LCC), and Chlorophyll *a*+*b* content were significant ($p \leq 0.05$) and positive at 30 and 60 days after planting under well-watered conditions. This indicates that LCC and CG, which provide estimates of leaf chlorophyll content, are effective indicators of leaf N status. In the current study, CG readings correlated positively with leaf retention (0.39**; $p \leq 0.01$) at DAY 60 under water stress, indicating a plausible relationship between leaf health and leaf water content under stress, but further research is needed to confirm this.

Canopy temperature is a function of leaf evaporative cooling due to open stomata, thus differences in leaf temperature of plants subjected to water stress can give us insight into genotypic differences in stomatal conductance. Relatively lower canopy temperature in drought stressed crop plants may indicate that soil moisture is still available and imply somewhat better plant water status. In general, leaf canopy temperature of cassava plants subjected to terminal water stress after 60 days was approximately double their counterpart suggesting stomatal closure during stress. Numerous studies have suggested that one of cassava's underlying mechanisms of tolerance to drought is its sensitive stomatal responses such as its stomatal closure under high evaporative demands even in well-watered conditions, and at mild levels of water scarcity (El-Sharkawy, 1993; El-Sharkawy, 2004; El-Sharkawy, 2006). In the current study, leaf ABA was negatively correlated with leaf-air temperature differential (*d*T) while it was positively correlated with soil water content at DAY 30 and DAY 60. A possible factor is that

ABA was sampled in the afternoon whereas *d*T was measured earlier in the day. Genotypes that tended to accumulate ABA in the afternoon may have conserved sufficient water (as indicated by the positive correlation between ABA and soil water content) so that in the morning at DAY 30 they had more open stomata and lower *d*T (as indicated by negative ABA correlation with *d*T). This suggestion is consistent with the finding that cassava readily closes its stomata in the afternoon (Cock et al., 1985; El-Sharkawy and Cock, 1984; El-Sharkawy et al., 1984; Itani et al., 1999) and it closes stomata in well watered conditions if air humidity and leaf-air vapor pressure difference is large (Cock, 1985; Connor and Palta, 1981).

Photosynthetic rates are drastically impaired during water stress to levels corresponding to the extent of stomatal closure, hindering subsequent biomass growth (Calatayud et al., 2000; El-Sharkawy, 2005; Itani et al., 1999). Our results are consistent with this: above ground biomass fresh weight, storage root fresh weight, number of storage roots, and partitioning index were drastically reduced after DAY 60 when compared to the well-watered controls. However, fibrous root fresh weight under stress was higher when compared to storage root fresh weight, suggesting the formation of root systems that may contribute to deep-water exploration in field soils (reviewed by El-Sharkawy, 2006). Likewise, leaf ABA was increased 2-fold under stress when compared to well watered controls by DAY 30. These results are in accordance with prior studies by Alves and Setter (2000 and 2004) and Duque and Setter (2005), which reported rapid ABA accumulation in response both to mild and severe water stress. However, in the current study, the plants receiving the WW treatment increased their level of leaf ABA at Day 60 such that ABA was about the same as controls. This was probably because although the WW treatment received daily irrigation, by Day 60 plant size was so large that transpiration used much of the pot water each day and soil moisture content prior to irrigation declined from about

0.17 at DAY 30 to about 0.09 $m^3 \times m^{-3}$ at DAY 60 (Fig. 2.3).

Regarding storage root fresh weight and yield components, aboveground biomass, fibrous root fresh weights, number of storage roots, and partitioning index, water stress reduced all components by DAY 60 when compared to controls. In the current study, partitioning index was decreased by 60% in WS and aboveground biomass by 65% compared to the WW treatment. In studies by (Aina et al., 2007), where field and screen house grown cassava was subjected to different levels of water stress in Nigeria, artificially imposed water stress was the largest contributor to loss in yield: plant height decreased by 47%, whereas number of storage roots decreased by 95%, and yield decreased by 87%.

Water stress also affected leaf non-structural carbohydrates when compared to controls. In general, leaf starch was depleted by DAY 30 (2-fold difference compared to well watered plants) as expected, due to reduced photosynthetic capacity in part because of partial stomatal closure. At DAY 60, water-stressed plants had a small but significant accumulation of leaf starch in fully expanded mature leaves suggestive of newly accumulated starch possibly due to extensive leaf abscission and photosynthates accruing in a few intact and attached leaves. On the other hand, leaf glucose and sucrose decreased slightly from DAY 30 to 60 under stress due to metabolic activity. Duque and Setter (2005) analyzed cassava non-structural carbohydrates in several plant parts. They suggested that cassava is capable of maintaining and conserving starch in its storage roots during a water stress episode, while its stem, which constitutes a storage organ for starch during non-stress conditions, becomes a source of slowly remobilized starch during stress. Fernandez et. al. (2002) evaluated the effects of elevated CO_2 and the lack of down-regulation of photosynthesis in cassava and found that soluble sugars and starch content decreased with time under elevated CO_2 concentrations, the decrease in starch content coinciding

with the beginning of the increase of root mass. The absence of down regulation of photosynthesis was associated with a decrease in leaf sugar and starch contents of plants grown in elevated CO_2, which suggests a favorable source/sink relationship. This physiological remobilization of starch reserve is suggested to be an additional drought tolerance mechanism against long periods of water stress.

The very strong phenotypic correlation observed between storage root fresh weight and partitioning index under stress (0.93**; $p \leq 0.01$) and in well-watered conditions (0.86**, $p \leq 0.01$) indicates that the storage root fresh weight differences observed under both water stress and well-watered conditions were mostly the result of a large difference in the capacity of cassava plants to maintain storage root growth under stress, rather than to differences in ability to accumulate above ground biomass. This indicates that selection for partitioning index at a relatively early stage of development should also result in an improvement in yield under both stress and well-watered conditions.

Other correlations with storage root fresh weight under stress, such as with difference in external temperature, plant height, leaf starch, and leaf ABA, indicated that these traits in conjunction with more detailed genetic studies could give insight into selecting for secondary traits (i.e. indirect selection) in association with yield under stress.

Genotypic correlations between several morpho-physiological traits (i.e., secondary traits) associated with drought tolerance and yield and yield components provide information on expected responses in yield and yield components from selection for these secondary traits. Genotypic correlations (r_G) for all traits measured under stress and control conditions are presented in Appendix Table A3. In general, correlations between SR_{FW} and morpho-physiological traits were variable and low. However, high and positive correlations were

observed between SR_{FW} and partitioning index (PI) under stress and in control conditions. The positive r_G under both environments suggests that it is possible to select genotypes combining drought tolerance with moderate to high yield in non-stress environments. However, the lower values of r_G also indicate that, in order to obtain such genotypes, selection should be performed in both stress and non-stress conditions. In addition, the r_G relationships between storage root fresh weight and partitioning index under both scenarios indicates that it is important that drought tolerance screening be conducted at stress levels that reduce yield substantially relative to a non-stress control, so that the stress can clearly differentiate a true drought-tolerant genotype from a genotype with a high-yield potential. The strong correlation observed between SR_{FW} and partitioning index under stress indicates that the yield differences observed under drought stress were mostly the result of a large difference in the capacity of plants to maintain storage root sink capacity under stress, rather than to accumulate biomass. This is consistent with conclusions reached in study of many other crops, especially those involving fruit, seed, and grain crops, where the flowering and early post-pollination phase is especially vulnerable to stress (Liu et al., 2006).

APPENDIX

Table A1. Analysis of variance for all traits measured on 45 cassava genotypes across water stress and well watered treatments at 30 and 60 days.

Trait	Unit	Sampling Date	Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
PH	cm	30	Model	89	66474.124	746.900	27.922	<.0001
			Error	360	9630	26.750		
			Corrected Total	449	76104.124			
			Genotype	44	44	63549.124	53.9925	<.0001
			Treatment	1	1	742.409	27.7536	<.0001
			Genotype×Treatment	44	44	2182.591	1.8544	0.0013
HFL	cm	30	Model	89	5372.658	60.367	5.5518	<.0001
			Error	360	3914.4	10.873		
			Corrected Total	449	9287.058			
			Genotype	44	44	3584.058	7.4913	<.0001
			Treatment	1	1	810.702	74.5588	<.0001
			Genotype×Treatment	44	44	977.898	2.044	0.0002
LR	%	30	Model	89	19679.041	221.113	4.4364	<.0001
			Error	360	17942.585	49.841		
			Corrected Total	449	37621.626			
			Genotype	44	44	11620.722	5.299	<.0001
			Treatment	1	1	3137.429	62.9494	<.0001
			Genotype×Treatment	44	44	4920.89	2.2439	<.0001
LCT	°C	30	Model	89	3766.978	42.326	11.142	<.0001
			Error	360	1367.552	3.799		
			Corrected Total	449	5134.53			
			Genotype	44	44	2445.387	14.6303	<.0001
			Treatment	1	1	657.877	173.1823	<.0001
			Genotype×Treatment	44	44	663.714	3.9709	<.0001
*d*T	°C	30	Model	89	6205.696	69.727	14.4486	<.0001
			Error	360	1737.304	4.826		
			Corrected Total	449	7943			
			Genotype	44	44	4536.696	21.3655	<.0001
			Treatment	1	1	690.928	143.1725	<.0001
			Genotype×Treatment	44	44	978.072	4.6062	<.0001
CG	SPAD	30	Model	89	12480.204	140.227	11.1217	<.0001
			Error	360	4539.04	12.608		
			Corrected Total	449	17019.244			
			Genotype	44	44	8527.083	15.3704	<.0001
			Treatment	1	1	2664.987	211.3652	<.0001
			Genotype×Treatment	44	44	1288.134	2.3219	<.0001
$\theta_{(0\text{-}5cm)}$	$m^3 \times m^3$	30	Model	89	1.252	0.014	23.3696	<.0001
			Error	360	0.217	0.001		
			Corrected Total	449	1.468			
			Genotype	44	44	0.076	2.8777	<.0001
			Treatment	1	1	1.135	1886.1152	<.0001
			Genotype×Treatment	44	44	0.04	1.5265	0.021
$\theta_{(20\text{-}25cm)}$	$m^3 \times m^3$	30	Model	89	1.229	0.014	13.7558	<.0001
			Error	360	0.361	0.001		
			Corrected Total	449	1.59			
			Genotype	44	44	0.196	4.4465	<.0001
			Treatment	1	1	0.918	914.0064	<.0001
			Genotype×Treatment	44	44	0.115	2.6048	<.0001

Table A1. (Continued)

Trait	Unit	Sampling Date	Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
PH	cm	60	Model	89	110196.231	1238.16	26.6589	<.0001
			Error	360	16720	46.444		
			Corrected Total	449	126916.231			
			Genotype	44	44	88948.031	43.5261	<.0001
			Treatment	1	1	16032.436	345.196	<.0001
			Genotype×Treatment	44	44	5215.764	2.5523	<.0001
HFL	cm	60	Model	89	164533.264	1848.688	78.3453	<.0001
			Error	360	8494.8	23.597		
			Corrected Total	449	173028.064			
			Genotype	44	44	26875.964	25.8857	<.0001
			Treatment	1	1	127310.58	5395.2781	<.0001
			Genotype×Treatment	44	44	10346.72	9.9655	<.0001
LR	%	60	Model	89	564126.619	6338.501	131.2078	<.0001
			Error	360	17391.19	48.309		
			Corrected Total	449	581517.809			
			Genotype	44	44	13560.938	6.3798	<.0001
			Treatment	1	1	543401.735	11248.4897	<.0001
			Genotype*Treatment	44	44	7163.946	3.3703	<.0001
LCT	°C	60	Model	89	4660.232	52.362	6.9153	<.0001
			Error	360	2725.885	7.572		
			Corrected Total	449	7386.117			
			Genotype	44	44	2392.887	7.1823	<.0001
			Treatment	1	1	1155.298	152.577	<.0001
			Genotype×Treatment	44	44	1112.046	3.3378	<.0001
*d*T	°C	60	Model	89	4593.28	51.61	11.4369	<.0001
			Error	360	1624.527	4.513		
			Corrected Total	449	6217.807			
			Genotype	44	44	3069.524	15.4594	<.0001
			Treatment	1	1	853.892	189.2249	<.0001
			Genotype×Treatment	44	44	669.864	3.3737	<.0001
CG	SPAD	60	Model	89	11891.614	133.614	7.5897	<.0001
			Error	360	6337.627	17.605		
			Corrected Total	449	18229.241			
			Genotype	44	44	9646.149	12.4531	<.0001
			Treatment	1	1	3.829	0.2175	0.6412
			Genotype×Treatment	44	44	2241.636	2.8939	<.0001
$\theta_{(0\text{-}5cm)}$	$m^3 \times m^3$	60	Model	89	0.698	0.008	10.744	<.0001
			Error	360	0.263	0.001		
			Corrected Total	449	0.96			
			Genotype	44	44	0.098	3.0437	<.0001
			Treatment	1	1	0.539	739.2612	<.0001
			Genotype×Treatment	44	44	0.061	1.8871	0.001
$\theta_{(20\text{-}25cm)60}$	$m^3 \times m^3$	60	Model	89	0.828	0.009	6.1927	<.0001
			Error	360	0.541	0.002		
			Corrected Total	449	1.369			
			Genotype	44	44	0.283	4.2773	<.0001
			Treatment	1	1	0.36	239.7055	<.0001
			Genotype×Treatment	44	44	0.185	2.801	<.0001

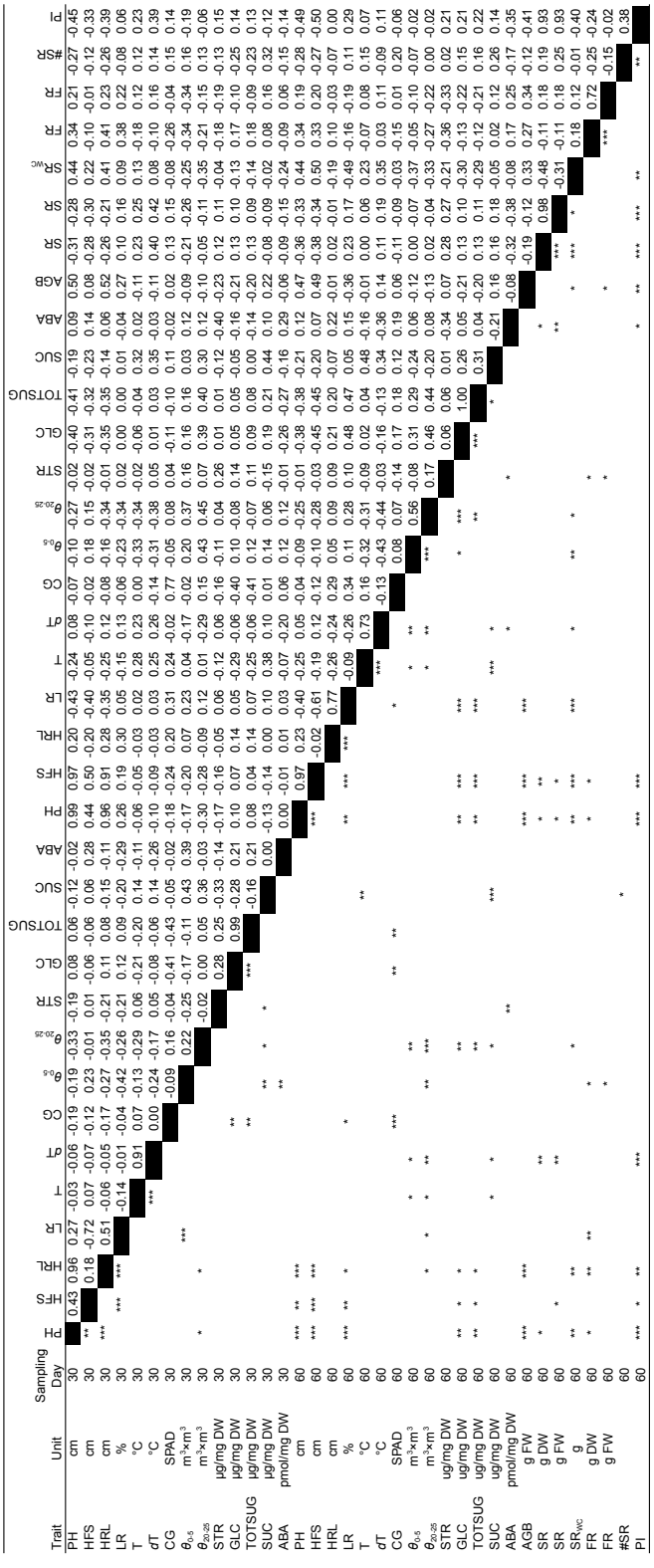
Table A1. (Continued)

Trait	Unit	Sampling Date	Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
AGB	g FW	60	Model	89	14303950	160719	12.8008	<.0001
			Error	360	4519920	12555		
			Corrected Total	449	18823871			
			Genotype	44	44	3374739.9	6.1088	<.0001
			Treatment	1	1	9087437.1	723.7909	<.0001
			Genotype×Treatment	44	44	1841773.1	3.3339	<.0001
SR	g FW	60	Model	89	1938224	21778	10.9258	<.0001
			Error	360	717565	1993		
			Corrected Total	449	2655790			
			Genotype	44	44	460480.7	5.2505	<.0001
			Treatment	1	1	1170246	587.1084	<.0001
			Genotype×Treatment	44	44	307497.7	3.5061	<.0001
SR	g DW	60	Model	89	172644	1940	8.2378	<.0001
			Error	360	84772	235		
			Corrected Total	449	257415			
			Genotype	44	44	45725.6	4.4133	<.0001
			Treatment	1	1	94084	399.547	<.0001
			Genotype×Treatment	44	44	32834.2	3.169	<.0001
FR	g FW	60	Model	89	505672	5682	8.9388	<.0001
			Error	360	228825	636		
			Corrected Total	449	734497			
			Genotype	44	44	226220.9	8.0887	<.0001
			Treatment	1	1	184777.7	290.7026	<.0001
			Genotype×Treatment	44	44	94673.1	3.3851	<.0001
FR	g DW	60	Model	89	19088	214	4.1347	<.0001
			Error	360	18674	52		
			Corrected Total	449	37762			
			Genotype	44	44	13006	5.6985	<.0001
			Treatment	1	1	1142.4	22.023	<.0001
			Genotype×Treatment	44	44	4939.7	2.1643	<.0001
#SR		60	Model	89	553	6	6.8106	<.0001
			Error	360	329	1		
			Corrected Total	449	882			
			Genotype	44	44	254.4	6.3338	<.0001
			Treatment	1	1	218.3	239.1013	<.0001
			Genotype×Treatment	44	44	80.7	2.008	0.0003
PI		60	Model	89	5	0	13.996	<.0001
			Error	360	1	0		
			Corrected Total	449	6			
			Genotype	44	44	2.5	14.3736	<.0001
			Treatment	1	1	1.8	458.6949	<.0001
			Genotype×Treatment	44	44	0.6	3.5116	<.0001

Table A1. (Continued)

Trait	Unit	Sampling Day	Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
STR	µg/mg DW	30	Model	89	1368319.3	15374.4	2.5662	<.0001
			Error	180	1078383	5991		
			Corrected Total	269	2446702.4			
			Genotype	44	44	503452.13	1.9099	0.0017
			Treatment	1	1	499552.55	83.3836	<.0001
			Genotype×Treatment	44	44	365314.63	1.3858	0.072
GLC	µg/mg DW	30	Model	89	113690.6	1277.4	2.1402	<.0001
			Error	180	107436.5	596.9		
			Corrected Total	269	221127.1			
			Genotype	44	44	92579.54	3.5252	<.0001
			Treatment	1	1	385.45	0.6458	0.4227
			Genotype×Treatment	44	44	20725.6	0.7892	0.8214
SUC	µg/mg DW	30	Model	89	110441.7	1240.9	2.1178	<.0001
			Error	180	105472.2	586		
			Corrected Total	269	215913.9			
			Genotype	44	44	89312.19	3.4641	<.0001
			Treatment	1	1	180.66	0.3083	0.5794
			Genotype×Treatment	44	44	20948.85	0.8125	0.7893
ABA	pmol/mg DW	30	Model	89	18959.5	213	9.582	<.0001
			Error	180	4001.8	22.2		
			Corrected Total	269	22961.3			
			Genotype	44	44	11696.55	11.9571	<.0001
			Treatment	1	1	4981.13	224.052	<.0001
			Genotype×Treatment	44	44	2281.82	2.3327	<.0001
LABA	pmol/mg DW	30	Model	89	36.7	0.4	12.7343	<.0001
			Error	180	5.8	0		
			Corrected Total	269	42.5			
			Genotype	44	44	21.4	15.0175	<.0001
			Treatment	1	1	12.63	389.997	<.0001
			Genotype×Treatment	44	44	2.67	1.8768	0.0022
STR	µg/mg DW	60	Model	89	669913.8	7527.1	1.3113	0.0646
			Error	180	1033272.2	5740.4		
			Corrected Total	269	1703186			
			Genotype	44	44	360880.54	1.4288	0.0551
			Treatment	1	1	79599.09	13.8665	0.0003
			Genotype×Treatment	44	44	229434.15	0.9084	0.6371
GLC	µg/mg DW	60	Model	89	196491.4	2207.8	5.3026	<.0001
			Error	180	74943.7	416.4		
			Corrected Total	269	271435.2			
			Genotype	44	44	54871.68	2.9952	<.0001
			Treatment	1	1	76814.17	184.492	<.0001
			Genotype×Treatment	44	44	64805.57	3.5375	<.0001
SUC	µg/mg DW	60	Model	89	196390.9	2206.6	5.2706	<.0001
			Error	180	75359.8	418.7		
			Corrected Total	269	271750.7			
			Genotype	44	44	57665.42	3.1304	<.0001
			Treatment	1	1	72040.78	172.072	<.0001
			Genotype×Treatment	44	44	66684.66	3.62	<.0001
ABA	pmol/mg DW	60	Model	89	15171.9	170.5	4.1255	<.0001
			Error	180	7437.9	41.3		
			Corrected Total	269	22609.8			
			Genotype	44	44	10559.4	5.8078	<.0001
			Treatment	1	1	229.91	5.5639	0.0194
			Genotype×Treatment	44	44	4382.59	2.4105	<.0001
LABA	pmol/mg DW	60	Model	89	19.1	0.2	6.698	<.0001
			Error	180	5.8	0		
			Corrected Total	269	24.9			
			Genotype	44	44	13.09	9.2847	<.0001
			Treatment	1	1	0.01	0.1637	0.6863
			Genotype×Treatment	44	44	6.01	4.2597	<.0001

Table A2. Phenotypic correlation estimates among all traits measured on 45 cassava genotypes exposed to terminal water-stressed (first table shown) and well-watered (second table shown) conditions measured at 30 and 60 days. *Significant at $p \leq 0.05$, **Significant at $P \leq 0.01$ and ***Significant at $P \leq 0.001$; (df = 45).



Trait	Unit	Sampling Day	PH	HFS	HRL	LR	T	dT	CG	$\theta_{0\text{-}5}$	$\theta_{20\text{-}25}$	STR	GLC	TOTSUG	SUC	ABA	PH	HFS	HRL	LR	T	dT	CG	$\theta_{0\text{-}5}$	$\theta_{20\text{-}25}$	STR	GLC	TOTSUG	SUC	ABA	AGB	SR	SR_{WC}	FR	#SR	PI
PH	cm	30		0.43	0.96	0.27	-0.03	-0.06	-0.19	-0.19	-0.33	-0.19	0.08	0.06	-0.12	-0.02	0.99	0.97	0.20	-0.43	-0.24	0.08	-0.07	-0.10	-0.27	-0.02	-0.40	-0.41	-0.19	0.09	0.50	-0.31	-0.28	0.44	0.34	0.21	-0.27	-0.45
HFS	cm	30	**		0.18	-0.72	0.07	-0.07	-0.12	-0.06	-0.01	0.01	-0.06	-0.06	0.06	0.28	0.44	0.50	-0.20	-0.40	-0.05	-0.10	-0.02	-0.02	0.15	-0.02	-0.31	-0.32	-0.23	0.14	0.08	-0.28	-0.30	0.22	-0.10	-0.01	-0.12	-0.33
HRL	cm	30	***			0.51	-0.06	-0.05	-0.17	-0.27	-0.35	-0.21	0.11	0.08	-0.15	-0.11	0.96	0.91	0.28	-0.35	-0.25	0.12	-0.08	-0.16	-0.34	-0.01	-0.35	-0.35	-0.14	0.06	0.52	-0.26	-0.21	0.41	0.41	0.23	-0.26	-0.39
LR	%	30		***	***		-0.14	-0.01	-0.04	-0.04	-0.26	-0.21	0.12	0.09	-0.20	-0.29	-0.26	-0.10	-0.09	0.30	0.05	-0.15	0.13	-0.06	-0.23	-0.34	0.02	0.00	0.00	0.01	-0.04	0.27	0.10	0.16	0.09	0.38	0.22	-0.08	0.06
T	°C	30						0.91	0.00	0.00	-0.17	0.05	-0.08	-0.06	0.14	0.00	-0.10	-0.09	-0.03	0.03	0.03	0.25	0.26	-0.14	-0.31	-0.38	0.05	0.01	0.03	0.35	-0.03	-0.11	0.40	0.42	0.08	-0.10	0.16	0.14	0.39
dT	°C	30					***		0.00	-0.09	0.16	-0.04	-0.41	-0.43	-0.05	-0.02	-0.18	-0.24	0.20	0.07	0.24	0.04	-0.17	-0.02	0.77	0.37	0.16	0.16	0.16	0.03	0.11	-0.02	0.02	-0.09	-0.26	-0.04	-0.34	0.16	0.14
CG	SPAD	30								-0.09	0.22	-0.25	-0.17	-0.11	0.43	0.39	-0.17	-0.20	0.07	0.23	0.04	-0.17	-0.02	0.20	0.37	0.45	0.07	0.39	0.40	0.30	-0.12	-0.40	-0.23	0.12	-0.04	-0.18	-0.19	0.15	-0.19
$\theta_{0\text{-}5}$	$m^3 \times m^{-3}$	30										-0.02	0.28	0.25	-0.33	-0.14	-0.17	-0.16	-0.05	0.06	-0.12	0.06	-0.16	-0.11	0.04	0.26	0.01	0.01	0.01	-0.12	-0.40	-0.23	0.12	0.11	-0.04	-0.19	-0.13	0.15
$\theta_{20\text{-}25}$	$m^3 \times m^{-3}$	30											0.99	0.99	-0.28	0.21	0.10	0.07	0.14	0.05	-0.29	-0.06	-0.40	0.10	-0.08	0.14	0.05	0.05	-0.05	-0.16	-0.21	0.13	0.10	-0.13	0.17	-0.10	-0.25	0.14
STR	µg/mg DW	30												0.99	-0.16	0.21	0.08	0.04	0.14	0.07	0.11	-0.06	-0.41	0.12	-0.07	0.11	0.09	0.08	0.00	-0.14	-0.20	0.13	0.09	-0.14	0.18	-0.09	-0.23	0.13
GLC	µg/mg DW	30													-0.16	0.21	0.10	0.07	0.14	0.05	-0.29	-0.06	-0.40	0.10	-0.08	0.14	0.05	0.05	-0.05	-0.16	-0.21	0.13	0.10	-0.13	0.17	-0.10	-0.25	0.14
TOTSUG	µg/mg DW	30														0.21	0.08	0.04	0.14	0.07	0.11	-0.06	-0.41	0.12	-0.07	0.11	0.09	0.08	0.00	-0.14	-0.20	0.13	0.09	-0.14	0.18	-0.09	-0.23	0.13
SUC	µg/mg DW	30															-0.13	-0.24	0.00	0.03	0.24	0.04	-0.17	-0.02	0.77	-0.14	-0.41	0.12	-0.07	0.00	-0.14	-0.20	0.13	0.09	-0.14	0.18	-0.09	-0.23	0.13
ABA	pmol/mg DW	30																-0.02	-0.09	0.12	0.01	-0.29	0.15	0.43	0.45	0.07	0.39	0.40	0.30	0.12	-0.40	-0.23	0.12	0.11	-0.04	-0.19	-0.13	0.15
PH	cm	60	***	**	***																																	
HFS	cm	60	**	***																																		
HRL	cm	60	***	***																																		
LR	%	60		*	*																																	
T	°C	60		**																																		
dT	°C	60													**																							
CG	SPAD	60																																			
$\theta_{0\text{-}5}$	$m^3 \times m^{-3}$	60						*	***				**	**																								
$\theta_{20\text{-}25}$	$m^3 \times m^{-3}$	60						**																														
STR	ug/mg DW	60	**										**	**			**																					
GLC	ug/mg DW	60	**	*	*									**			**	***	***																			
TOTSUG	ug/mg DW	60	**	*	*												**	***	***																			
SUC	ug/mg DW	60						*							***						***																
ABA	pmol/mg DW	60										**																										
AGB	g FW	60	***		***												***	***		***																		
SR	g DW	60	*					**									*	**																				
SR	g FW	60		*				**									*	*																				
SR_{WC}	g	60	**		**												**	***		***																		
FR	g DW	60	*		**	**											*																					
FR	g FW	60																																				
#SR		60	***	*	**			***									***	***																				
PI		60																																				

Table A2. (Continued)

Table A3. Genotypic correlation estimates among several traits of 45 cassava genotypes exposed to terminal well-watered (above diagonal in italics) and water-stressed (below diagonal) conditions measured at 30 and 60 days.*Significant at $p \leq 0.05$. **Significant at $p \leq 0.01$. (df = 45). ns = non significant. Genetic correlations were calculated as described in Eq. [3].

	Unit	Sampling Day	PH	LR	T	dT	CG	$\theta_{0.5}$	$\theta_{0.0.25}$	PH	LR	T	dT	CG	$\theta_{0.5}$	$\theta_{0.25}$	AGB	SR	FR	#SR	PI	STR	GLC	SUC	ABA	STR	GLC	SUC	ABA
PH	cm	30		-0.07	-0.04	-0.07	0.02	-0.25	-0.48 **	0.99 **	-0.16 *	-0.08	0.15	0.26	-0.29	-0.46 **	0.69 **	-0.08	0.34 *	-0.34 *	-0.43 **	ns	-0.45 **	-0.47 **	-0.69 **	-0.58 **	-0.48 **	-0.49 **	-0.51 **
LR	%	30	0.33		-0.04	0.07 **	-0.08	0.12	-0.26	0.02	0.79 **	-0.35 *	-0.33 *	-0.04	-0.01	-0.27	0.26	0.28	0.05	0.11	0.06	ns	-0.62 **	-0.61 **	ns	ns	-0.56 **	-0.57 **	-0.34 *
T	°C	30	-0.03	-0.18		0.81 **	-0.13	-0.16 *	0.03	-0.04	-0.25	0.46 **	0.43 *	0.12	-0.29	0.01	0.08	-0.06	0.25	0.00	-0.05	ns	ns	ns	-0.59 *	ns	ns	-0.58 *
dT	°C	30	-0.06	-0.01	0.91 **		-0.25	-0.31 *	0.05	-0.08	-0.24	0.42 *	0.56 **	0.10 **	-0.29	-0.21	0.13	0.09	0.13	0.15	0.05	ns	-0.70 **	-0.73 **	-0.47 *	ns	ns	ns	-0.50 *
CG	SPAD	30	-0.21	-0.06	0.08	0.00		0.51 **	0.07	0.03	0.02	0.32 *	-0.17	0.69 **	-0.05	0.08	-0.01	0.03	0.18	-0.17	-0.03	ns	-1.00 **	ns	-0.30 *	-0.73 **	-0.72 **	-0.76 **	-0.22
$\theta_{0.5}$	$m^3 \times m^3$	30	-0.30 *	-0.77 **	-0.24	-0.36 *	-0.18		0.26	-0.17	0.23	0.14	-0.53 **	0.06	0.23	0.59 **	-0.29	-0.27	-0.03	-0.15	-0.04	ns	ns	ns	ns	ns	ns	ns	ns
$\theta_{0.0.25}$	$m^3 \times m^3$	30	-0.43 **	-0.46 **	-0.37 *	-0.20	0.23	0.31 *		-0.50 **	-0.15	0.09	0.02	-0.27	0.48 **	0.49 **	-0.41 **	-0.34 *	-0.28	-0.23 *	-0.04	ns	ns	ns	ns	ns	ns	ns	ns
PH	cm	60	1.00 **	0.31 *	-0.06	-0.10	-0.20	-0.27 *	-0.39 *		-0.44 **	-0.10	0.12	0.29	-0.25	-0.44 **	0.70 **	-0.12	0.32 *	-0.39 **	-0.46 **	ns	-0.42 **	-0.43 **	-0.78 **	-0.50 **	-0.45 **	-0.46 **	-0.60 **
LR	%	60	0.47 **	0.01	0.01 *	0.02 *	0.32 *	0.38 **	0.15	-0.44 **		-0.07	-0.54 **	-0.13	0.21	0.08	0.08	0.07	0.03	-0.15	0.03	ns	-0.39 *	-0.40 *	0.00 *	-0.87 **	-0.77 **	-0.78 **	-0.15 *
T	°C	60	-0.39 **	-0.19	0.35 *	0.35 *	0.32 *	0.15 *	0.03 *	-0.42 **	0.09		-0.26 **	0.68 **	-0.12 *	-0.04 **	-0.17 *	-0.02 *	0.16 *	-0.09 *	0.07	ns	ns	ns	-0.47 **	ns	ns	ns	-0.57 **
dT	°C	60	0.05	0.20	0.29	0.36 *	0.02	-0.30 *	-0.41 *	0.00 *	-0.27	0.68 **		0.36 **	-0.34 *	-0.68 **	0.29 *	0.11	0.00	-0.03 *	-0.06	ns	-0.77 **	-0.84 **	-0.72 **	ns	ns	ns	-0.64 **
CG	SPAD	60	-0.11	-0.06	-0.05	-0.18	0.84 **	-0.03	0.18	-0.08	0.41 **	0.17	-0.22 *	0.29	-0.42 **	-0.45 **	0.40 **	0.08	0.16 *	-0.21 *	-0.19	ns	-0.87 **	-0.88 **	-0.45 **	-0.98 **	-0.73 **	-0.76 **	-0.28
$\theta_{0.5}$	$m^3 \times m^3$	60	-0.17	-0.33 *	-0.49 **	-0.41 **	-0.12	0.60 **	0.63 **	-0.15	0.17	-0.42 **	-0.67 **	0.07	1.00 **	0.77 **	-0.60 **	-0.09 *	-0.44 **	0.13	0.23	ns	ns	ns	ns	ns	ns	ns	ns
$\theta_{0.0.25}$	$m^3 \times m^3$	60	-0.35 *	-0.48 **	-0.45 **	-0.46 **	0.12	0.65 **	0.68 **	-0.32 *	0.35 *	-0.50 **	-0.64 **	0.06		-0.73 **	-0.09	-0.09	-0.41 **	0.14 **	0.30 *	ns	ns	ns	ns	ns	ns	ns	ns
AGB	g FW	60	0.54 **	0.40 **	-0.12	-0.13 *	0.03	-0.13 *	-0.16	0.52 **	-0.40 **	0.00	0.23 *	0.11	-0.17		0.08	-0.20	0.56 **	-0.49 **	0.60 **	ns	ns	ns	ns	ns	ns	ns	ns
SR	g FW	60	-0.30 *	0.20 *	0.27	0.45 **	0.17	-0.37 *	-0.18	-0.35 *	0.19	0.21	0.33 *	-0.09	-0.18	-0.07 *	-0.16 *		-0.33 *	0.84 **	-0.67 **	-0.62 **	-0.72 **	-0.69 **	ns	-0.76 **	-0.67 **	-0.66 **	ns
FR	g FW	60	0.23 *	0.33 *	0.18 *	0.22 *	-0.02	-0.51 **	-0.17	0.21 *	-0.19 *	0.17 *	0.19 *	0.05	-0.22 *	-0.33 *	0.35 *	0.18 *		-0.45 **	-0.56 **	-0.43 **	-0.95 **	-0.95 **	ns	-0.66 **	-0.47 **	-0.46 **	ns
#SR		60	-0.31 *	-0.09	0.12	0.15 *	0.19	0.29	0.25	-0.32 *	0.13 *	0.29	-0.09 *	0.24	-0.14 *	-0.01	-0.17 *	0.23 **	-0.22 *		0.74 **	ns	ns	ns	ns	ns	ns	ns	ns
PI		60	-0.47 **	0.05	0.25 *	0.43 **	0.16 *	-0.32 *	-0.05	-0.52 **	0.32 *	0.24 *	0.22 *	-0.06 *	-0.04 *	-0.11 *	-0.44 **	0.94 **	-0.04 *	0.39 **		ns	ns	ns	ns	ns	ns	ns	ns
STR	µg/mg DW	30	-0.64 **	-0.74 **	-0.81 **	-0.65 **	-0.68 **	ns	ns	-0.63 **	-0.45 **	ns	-0.90 **	-0.72 **	ns	ns	-0.74 **	-0.24 *	-0.58 **	ns	ns	-0.13	ns	ns	ns	-0.26 *	-0.41 **	-0.40 **	ns
GLC	µg/mg DW	30	-0.37 *	-0.57 **	ns	-0.99 **	ns	ns	ns	-0.34 *	-0.56 **	ns	ns	ns	ns	ns	-0.57 **	-0.38 **	-0.49 **	ns	ns	-0.16 **	0.20 *	0.19 *	-0.62 **	-0.45 **	-0.63 **	-0.61 **	-0.79 **
SUC	µg/mg DW	30	-0.38 **	-0.56 **	ns	-0.94 **	ns	ns	ns	-0.35 *	-0.52 **	ns	-0.83 **	ns	ns	ns	-0.82 **	-0.38 **	-0.46 **	ns	ns	-0.56 **	-0.39 **	-0.37 *	-0.58 **	-0.44 **	-0.61 **	-0.59 **	-0.74 **
ABA	pmol/mg DW	30	-0.40 **	-0.55 **	-0.71 **	-0.88 **	-0.45 **	ns	ns	-0.37 *	-0.39 *	-0.73 **	-0.79 **	-0.40 **	ns	ns	-0.15 *	0.38 **	ns	ns	ns	0.22 *	0.14 *	0.15 *	-0.34 *	ns	-0.76 **	-0.78 **	0.02 **
STR	µg/mg DW	60	-0.69 **	ns	-0.78 **	-0.40 **	-0.29 *	ns	ns	-0.75 **	0.38 *	-0.82 **	ns	ns	ns	ns	-0.54 **	-0.41 **	-0.63 **	ns	ns	-0.61 **	-0.36 **	-0.32 *	-0.69 **	0.04	-0.54 **	-0.56 **	-0.98 **
GLC	µg/mg DW	60	-0.73 **	-0.68 **	ns	ns	-0.89 **	ns	ns	-0.71 **	-0.27	ns	ns	-0.57 **	ns	ns	-0.53 **	-0.40 **	-0.63 **	ns	ns	-0.62 **	-0.36 **	-0.32 *	-0.70 **	0.00	0.20	-0.78 **	
SUC	µg/mg DW	60	-0.74 **	-0.70 **	ns	ns	-0.90 **	ns	ns	-0.72 **	-0.29	ns	ns	-0.59 **	ns	ns	-0.53 **	-0.40 **	-0.63 **	ns	ns	-0.62 **	-0.36 **	-0.32 *	-0.70 **	0.00	0.20		-0.78 **
ABA	pmol/mg DW	60	-0.36 *	-0.37 *	-0.67 **	-0.55 **	-0.50 **	ns	ns	-0.32 *	-0.26	-0.77 **	-0.93 **	-0.20	ns	ns	-0.85 **	-0.63 **	-0.25	ns	ns	-0.98 **	-0.62 **	-0.59 **	-0.18	ns	-0.53 **	-0.54 **	

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CHAPTER 3:

Genotypic analysis of cassava drought tolerance traits: Heritability and genotype × environment interaction

3.1 Introduction

Cassava (*Manihot esculenta* Crantz) is the third most important source of calories in the tropics, after rice and maize, and fourth worldwide (Cock, 1982; FAO, 2007; Hillocks, 2002; Nweke, 2004). Production was 241 million tons (fresh root equivalent; 134 million tons dry basis) in 2009 (FAOSTAT, 2011). It is an important staple food crop for nearly half a billion people in developing countries of the tropics and subtropics, especially in Sub-Saharan Africa. Countries in sub-Saharan Africa obtain a high percentage of food energy from cassava, especially those with low per-capita caloric intake such as DR Congo (56% of calories derived from cassava consumption), Mozambique (36%), Republic of Congo (31%), and Angola (31%) (Takeshima, 2010). Cassava is cultivated by subsistence farmers in areas considered marginal for other crops. Thus it provides a livelihood for millions of farmers, processors, and traders worldwide (Cock, 1982; El-Sharkawy, 2006; Hillocks, 2002). Because of its tolerance to drought, ability to survive unreliable rainfall patterns and broad agro-ecological adaptability, it is considered a contributor to food security against famine, providing reliable yields with minimal inputs in tropical and sub-tropical Africa, Asia, and Latin America (El-Sharkawy, 2004; El-Sharkawy, 2006; Setter and Fregene, 2007).

Cassava is valued for its stable yield performance in stress-prone environments (El-Sharkawy, 2006; El-Sharkawy et al., 1993; Hillocks, 2002; Nweke, 2004). However, it is also

one of the most productive crops available for favorable environments. Farmers prefer genotypes that combine both stress tolerance to ensure an adequate harvest in years with poor weather, and genotypes which also have high yield potential in years with favorable weather. Thus, it is important to evaluate genotype × environment interaction of cassava genotypes to identify traits that confer good yields in a range of environments. Breeding for improved drought resistance can increase long-term productivity in drought prone regions (Setter and Fregene, 2007). Attempts to develop high yielding and drought tolerant varieties are currently being encouraged (El-Sharkawy, 2006; El-Sharkawy et al., 1993; Hillocks, 2002; Nweke, 2004).

Several breeding approaches have been proposed that involve evaluation of physiological traits to improve the efficiency of selection for superior drought-tolerant genotypes (Richards et al., 2010). Many researchers have also studied the physiological and molecular aspects of drought tolerance and the manner in which these traits have been integrated as selection tools in many plant breeding programs worldwide (Blum, 2005; Bruce et al., 2002; Landi et al., 2007; Tuberosa and Salvi, 2006; Tuberosa et al., 2002; Tuberosa et al., 2003; Zhao et al., 2008a; Zhao et al., 2008b; Zhao et al., 2008c). In spite of this, the occasional use of certain technologies and/or traits have been unsuccessful because specified traits have been considered in isolation, often unrelated to superior performance under drought stress. In addition, the timing and duration of water stress can also play a crucial role in the success or failure in selecting elite drought-tolerant genotypes for specific environments. Many plant breeders and crop physiologists believe more rapid progress can be obtained by pre-breeding research to develop knowledge of the physiological basis of crop performance under drought conditions. One strategy for use of this knowledge involves the breeding of better adapted and higher-yielding cultivars by identifying reliable secondary traits of drought-tolerance, such as morphological and/or physiological

characters, to complement and simplify conventional breeding programs (Bidinger and Witcombe, 1989; Fukai et al., 1999; Ober et al., 2005).

Secondary traits are plant characteristics that are associated with yield, the primary trait, which provide additional information to plant breeders when they make selections. Plant breeders who select for disease scores, plant height, and flowering date, as well as many other traits, are all using secondary traits (Fischer et al., 2003). For the "indirect" or "secondary trait" approach to be successful, the trait must have high genetic correlation with yield, high heritability, be easily measurable, provide an estimate of yield potential, and the selection method must be applicable on a large scale (Richards et al., 2001). Valuable secondary or indirect traits are assumed to be under simpler genetic control than yield because they involve a subset of plant function whereas yield depends on a much larger set of functions. Nevertheless, to be used for crop improvement, the secondary trait must be highly correlated with yield (Kumar et al., 2007).

The use of secondary traits has the potential to improve response to selection by focusing on traits with relatively high heritability and with direct relevance to drought tolerance, and by avoiding confounding factors such as additional stresses (e.g., soil fertility, micronutrient deficiency, and pathogens) that also determine final yield (Monneveux and Ribaut, 2006; Monneveux et al., 2006). To assess whether this approach has potential for cassava, variance components and heritability of secondary traits need to be determined and the association of these traits to yield needs to be evaluated on the basis of controlled field experiments.

Secondary traits have been used for improving drought tolerance in several crops. Notable examples of the success that can be achieved are illustrated by secondary traits in maize and wheat. In maize, anthesis silking interval (ASI) is a trait that is easy and relatively

inexpensive to score in managed stress trials (1996). The trait has a relatively high heritability and it correlates strongly with grain yield (Banziger and Lafitte, 1997). Selection for ASI has been successfully used to improve drought tolerance of breeding populations and to develop elite drought tolerant hybrids for the tropics (Ribaut et al., 2009). In wheat, low $\Delta^{13}C$ isotope ratio, a secondary trait with high correlation to water use efficiency (WUE), has been used to develop improved lines in Australia (Condon et al., 2004). Although the analysis of isotope abundance is expensive, the trait has a heritability that is higher than yield, and selection for high WUE has been successfully achieved.

In plant breeding, the response to phenotypic selection over all environments is the product of selection intensity, the additive part of broad-sense heritability and σ_p (phenotypic standard deviation). Heritability is reduced by genotype × environment (G × E) interactions and is thus a key attribute with respect to genetic gains for crops that will be subjected to uncertain environmental conditions and some probability of stress.

The current chapter will examine several traits in cassava grown in contrasting environments of either water stress or well-watered conditions for their potential use as secondary traits in a breeding program. The goals in this work were to phenotype a population of diverse cassava genotypes under water stress and 1) estimate heritability (H^2) for a range of prospective secondary traits, 2) correlate genotypic data of several secondary traits against storage root yield, and 3) assess which secondary traits have potential for use in breeding programs for drought tolerance.

3.2 Materials and Methods

This study was carried out during the dry season from May to August 2007 at the International Center for Tropical Agriculture (CIAT), located at the outskirts of the city of Palmira, Colombia. Forty-five contrasting cassava genotypes were chosen by cassava breeders from CIAT and EMBRAPA-CNPMF (Brazil) and used to determine the effects of terminal artificially induced water stress on several morpho-physiological traits.

Approximately 20 stem cuttings (25-30 cm long) of each genotype were disinfected and sown in 2 kg plastic bags containing sterilized mineral soil : coarse sand (2:1), placed in an outside nursery and received constant manual irrigation. After 60 DAP, ten randomly selected plants of each genotype were transplanted into 50 kg plastic bags containing the same soil mixture as described above and watered to field capacity. From these ten plants, five plants were randomly chosen to represent the well-watered (WW) environment and the remaining plants to represent the water-stressed (WS) environment. All WS plants were covered with a transparent plastic lining placed and sealed at the base of the stem of each cutting and over the rim to prevent percolation of naturally occurring rainwater. Afterwards, all plants were taken to the experimental field and randomly placed in grid fashion (1 m ×1 m). At this stage, referred to as DAY 0, manual irrigation was withheld for WS plants and WW plants were supplied manual irrigation as needed. Two environments were created: (i) control (plants were irrigated every other day until drainage occurred) and (ii) water stress (irrigation was withheld and soil was allowed to dry for the duration of the experiment).

The mean maximum and minimum air temperature during the experimental period ranged between 40 °C and 15 °C. The mean dew point was at 20 °C. Relative humidity oscillated

between 100% and 15%. The driest months are June, July, and, August with a monthly mean precipitation of 55, 28, and 46 mm. The annual mean precipitation was 908 mm. In addition, the annual mean solar radiation is 4.5-5.0 KwH m^{-2} day^{-1} with an annual mean sunlight between 1700-2100 hours. The annual potential evapotranspiration for this region is 1343 mm. The first sampling date was taken 30 days after the onset of drought (DAY 30). The second and final sampling date was taken at DAY 60. At DAY 60 all plant material was harvested and yield components evaluated.

At each sampling date, a number of morpho-physiological traits were recorded. Plant height (PH) was measured as the distance from the soil surface (main stem base) to the uppermost fully expanded leaf. Leaf retention (LR) was calculated from the following expression:

[1] $$LR(\%) = \frac{HRL}{PH} \times 100$$

where *HRL* is the height of the stem containing retained leaves. This procedure was adopted in order to insure uniformity between all genotypes. Leaf canopy temperature (T) and difference in external temperature (dT) were measured using a handheld infrared thermometer (model AG-42D, Telatemp Corporation, U.S.A.). Leaf chlorophyll greenness (CG) was measured using a Minolta SPAD-502M chlorophyll meter (Tokyo, Japan), volumetric soil water content (θ) was assessed at two different soil depths (0-5 cm and 20-25 cm) using a ThetaProbe Soil Moisture Sensor (model ML2x; Delta-T Devices, UK). Yield components measured at DAY 60 only were: aboveground biomass (AGB_{FW}), storage root fresh weight (SR_{FW}), fibrous root fresh weight (FR_{FW}), number of storage roots (#SR), and fresh weight partitioning index (*PI*).

Partitioning index was measured as the ratio between storage root fresh weight and total biomass expressed in the following equation:

[2] $$PI = \frac{SR_{FW}}{AGB_{FW} + FR_{FW} + SR_{FW}} \times 100$$

Leaf abscisic acid (ABA) and non-structural carbohydrates [sucrose (SUC), glucose (GLC), and starch (STR)] were measured using an enzyme-linked immunosorbant assay (ELISA) and the peroxidase/glucose oxidase (PGO) method similar to Ober et. al. (1991) and Setter et. al. (2001).

All data was subjected to analysis of variance, using a JMP 7.0 statistical package (SAS Institute, Inc. U.S.A.). The significance of the factor effect was determined using the F-test.

For broad sense heritability (H^2) estimates, variance components were estimated for a model in which all factors were considered fixed (genotypes and environmental effects). The model for combined analysis over the watering environments (WW and WS) had as sources of variation: genotype (G), watering environment (E), and G × E. Broad sense heritability (H^2) was calculated using the combined data as:

[3] $$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + (\sigma_{GE}^2 / \eta) + [\sigma_e^2 / (r\eta)]}$$

where σ_G^2, σ_{GE}^2, and σ_e^2 are the genotypic, G×E, and residual variances, respectively, and η and *r* are the number of environments and replicates, respectively (Cooper and Delacy, 1994; Cooper et al., 1996).

For separate analysis of each watering environment, a model was used which included genotypic and residual sources of variation, and H^2 was calculated as:

[4] $$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + [\sigma_e^2 / r]}$$

Where σ_G^2 and σ_e^2 are the genotypic and residual variance components, respectively, and *r* is the number of replicates(Cooper et al., 1996). This estimator of broad-sense heritability (H^2) is biased upward by any variance due to genotype × environment interaction, which was not partitioned away from genotypic variance. Single-environment H^2 was used to approximately compare all traits at each watering environment, and to calculate genotypic correlations in the combined analysis of environments, as described below.

Genetic correlations between the two watering environments were calculated as:

[5] $$r_{G(jj')} = \frac{r_{P(jj')}}{\sqrt{H_j^2 \times H_{j'}^2}}$$

(Cooper and Delacy, 1994; Cooper et al., 1996) where $r_{G(jj')}$, $r_{P(jj')}$, H_j^2, and $H_{j'}^2$ are genotypic correlation between environments j and j', phenotypic correlation between the same pair of

environments, and H^2 of traits evaluated for environments j and j', respectively. Phenotypic correlations were computed based on genotype means in each environment.

Genotypic correlations between two traits, 1 and 2, in the same environment were calculated as:

[6] $$r_{G12} = \frac{Cov_{12}}{\sqrt{\sigma^2_{G1} \times \sigma^2_{G2}}}$$

(Bernardo, 2002; Bernier et al., 2007; Kumar et al., 2007)where r_{G12}, Cov_{12}, $\sigma^2_{G1,}$ and $\sigma^2_{G2}$are the genetic correlation coefficient between traits 1 and 2 within the same environment, genetic covariance of traits 1 and 2, and the genotypic variances of traits 1 and 2, respectively. This estimation method assumes that the covariance between genotype means is entirely caused by correlation of genotypic effects and that there are no environmental effects apart from the uncontrolled residual effects between replicate plants. Genetic correlations were reported only when the phenotypic correlation between the two traits was significant ($P \leq 0.05$). In a recent study by Kumar et al. (2007), resulting covariance and variance component estimates had large sampling errors, thus estimates of r_G were imprecise. Hence, these results should be considered as rough guides to the degree of genetic association between traits. Genetic correlations estimated from linear functions of covariances tend to "over-correct" phenotypic correlations when H^2 for one or both of the traits involved is very low, resulting in estimates substantially greater than 1 or less than -1 (Kumar et al., 2007). As a result, genetic correlations for such traits were omitted in this experiment.

To further analyze the G × E interaction, which negatively affects heritability and response to selection, G × E was partitioned into two components, (i) heterogeneity of genotypic variance across environments, and (ii) lack of genetic correlation across environments (Cooper and Delacy, 1994; Cooper et al., 1996). The sum of these two components equals the G × E variance. Heterogeneity of genotypic variance across two environments was calculated as:

[7] $$V(\sigma_{G(env)}) = \frac{(\sigma_{G(j)} - \sigma_{G(j')})^2}{n_e(n_e - 1)}$$

where $\sigma_{G(j)}$ and $\sigma_{G(j')}$ are the square roots of genotypic variance in environments j and j', respectively, as defined in the single environment model, and n_e is the number of environments. The component of G × E interaction due to lack of correlation across the two environments was calculated as:

[8] $$L(r_{G(env)}) = \frac{2(\sigma_{G(j)}\sigma_{G(j')}(1 - r_{G(jj')})}{n_e(n_e - 1)}$$

where $r_{G(jj')}$ is the genotypic correlation between the two environments, as calculated above, $\sigma_{G(j)}$ and $\sigma_{G(j')}$ are the square roots of genotypic variance as described above, and n_e is the number of environments included in the analysis (two).

3.3 Results

3.3.1 Effects on variance components and heritability

The main purpose of estimating the heritability and variance components that constitute heritability estimates is to compare the expected gains from selection based on alternative selection strategies and different environments. For example, heritability estimates can be used to predict gain from selection based on single, unreplicated plot values, and compare this to gain from selection estimated if genotypes are replicated within and across macroenvironments (Hoi et al., 1999).

Estimates of genotypic variance and broad sense heritability were made for all traits measured under stress and well-watered conditions (Table 3.1). Overall, genotypic (σ^2_G) and residual (σ^2_e) variance components between both environments were of similar magnitude for plant height (PH), leaf retention (LR), leaf chlorophyll greenness (CG), and volumetric soil water content (θ) at DAY 30. For DAY 60, variance components were similar in water stress and well watered environments for leaf retention, leaf canopy temperature (T), difference in external temperature (dT), leaf chlorophyll greenness, and volumetric soil water content. In contrast, variance in well watered compared to water-stressed environments for aboveground biomass and weight of plant parts at final harvest were extremely different, in some cases differing by several orders of magnitude. This was because water stress severely slowed plant growth such that the range and residual variance for plant biomass in stress plants was relatively small. Abscisic acid (ABA) variance components were also dissimilar between the environments, although in this case the average values and residual variances were much smaller in the well-watered controls.

Heritabilities estimated using the single-environment model were high (exceeding 0.70) for most biomass and morphology traits in both environments (Table 3.1). They were also high

for canopy temperature and leaf ABA in both environments, and in soil water content and leaf sugar in the well-watered environment. In contrast, heritabilities were low for leaf starch, particularly at DAY 30 (STR_{30}) and DAY 60 (STR_{60}) under stress (0.29 and 0.02, respectively). In general, heritabilities for sugars were relatively low (<0.70) in water stress, and for glucose and total sugar on DAY 30. Heritability for volumetric soil water content (θ) was relatively low (between 0.43 and 0.74), with apparently higher values in well watered than water stressed conditions at both depths and at both sampling dates. Regarding traits related to growth and biomass accumulation, heritability of plant height was high (≥0.95) at both Day 30 and 60, and in both water stressed and well watered environments, and heritability for fresh weight partitioning index was high (≥0.88) in both environments. Heritability of fibrous root fresh weight (FR_{FW}) was slightly higher in well-watered conditions when compared to water-stressed conditions, whereas storage root heritabilities were higher in the water stressed than well-watered environment. In general, heritabilities were similar in well watered and stress environments despite the substantial stress effects on growth, soil water content, canopy temperature, leaf retention, and metabolite levels, as described in Chapter 2. These high heritabilities in water stress might be a reflection of the tight control of stress that was possible in this managed-stress experiment.

Table 3.1 Variance components and heritabilities for traits measured under water stress and well-watered conditions in 45 cassava genotypes at two sampling dates. For each genotype-sampling date combination, five replicates of biomass and non-destructive physiological traits, and three replicates of carbohydrate and ABA traits were measured. Significance of genotypic effects are indicated symbolically with ‡, *, **, and *** for P< 0.10, 0.05, 0.01, and 0.001, respectively. ns = non significant. *SS* = sum of squares. *d.f.* = degrees of freedom. Genotypic variance (σ^2_G), error variance (σ^2_e). Heritability for each environment was calculated as described in Eq. [3].

			Well-watered						Water-stressed					
Trait	Unit	Sampling Day	*SS*	*d.f.*	*Sig.*	σ^2_G	σ^2_e	H^2	*SS*	*d.f.*	*Sig.*	σ^2_G	σ^2_e	H^2
AGB	g FW	60	4968499	44	***	17743.40	24203	0.79	248014	44	***	945.8	908	0.84
SR	g DW	60	75699	44	***	251.60	462	0.73	2859.9	44	***	11.34	8.3	0.87
SR	g FW	60	738999	44	***	2575.40	3918	0.77	28755.8	44	***	117.5	66	0.90
SR_{wc}	g	60	0.5	44	***	0.0016	0.0035	0.69	0.906	44	***	0.003	0.0058	0.72
FR	g FW	60	281018	44	***	1061.20	1081	0.83	39876	44	***	143.2	190	0.79
FR	g DW	60	16195	44	***	55.38	91.2	0.75	1750.71	44	***	5.45	12.55	0.68
#SR		60	210.86	44	***	0.71	1.22	0.74	127.45	44	***	0.4576	0.609	0.79
PI		60	2.22	44	***	0.0089	0.0061	0.88	0.93	44	***	0.0039	0.0019	0.91
PH	cm	30	33509	44	***	146.60	29	0.96	32223	44	***	141.4	25	0.97
PH	cm	60	60766	44	***	262.60	68	0.95	33398	44	***	146.8	25	0.97
HFS	cm	30	2134.64	44	***	7.43	11.38	0.77	2328.96	44	***	8.514	10.36	0.80
HFS	cm	60	5393.4	44	***	19.96	22.8	0.81	31829	44	***	139.8	24	0.97
HRL	cm	30	28576.5	44	***	123.18	33.6	0.95	27069.8	44	***	118.02	25.1	0.96
HRL	cm	60	46924	44	***	194.20	95	0.91	2084.36	44	***	7.8	8.38	0.82
LR	%	30	8109.6	44	***	25.62	56.2	0.70	7693.2	44	***	26.26	43.5	0.75
LR	%	60	11297.4	44	***	37.62	68.7	0.73	9427.1	44	***	37.28	27.9	0.87
CG	SPAD	30	4854.7	44	***	19.58	12.4	0.89	4960.5	44	***	19.98	12.8	0.89
CG	SPAD	60	5467.2	44	***	20.32	22.7	0.82	6360	44	***	26.3	13	0.91
T	°C	30	1184	44	***	4.54	4.21	0.84	1925.11	44	***	8.07	3.39	0.92
T	°C	60	1592.21	44	***	6.32	4.61	0.87	2400.69	44	***	9.31	8.02	0.85
*d*T	°C	30	2215.59	44	***	8.99	5.41	0.89	3299.2	44	***	14.16	4.2	0.94
*d*T	°C	60	1697.44	44	***	7.07	3.23	0.92	3002.96	44	***	12.81	4.21	0.94
$\theta_{0\text{-}5}$	$m^3 \times m^3$	30	0.09	44	***	0.0002	0.0009	0.57	0.0253	44	**	0.00005	0.0003	0.43
$\theta_{0\text{-}5}$	$m^3 \times m^3$	60	0.1	44	***	0.0003	0.0007	0.70	0.0606	44	**	0.0001	0.0007	0.47
$\theta_{20\text{-}25}$	$m^3 \times m^3$	30	0.25	44	***	0.0008	0.0015	0.74	0.0551	44	***	0.0002	0.0005	0.62
$\theta_{20\text{-}25}$	$m^3 \times m^3$	60	0.42	44	***	0.0014	0.0026	0.73	0.0460	44	***	0.0001	0.0004	0.66
STR	µg/mg DW	30	803080	44	*	2443	10923	0.40	65686	44	‡	144.67	1059	0.29
STR	µg/mg DW	60	211856	44	*	583.33	3065	0.36	360446	43	ns	-75	8607	-0.03
GLC	µg/mg DW	30	54217	44	*	172.67	714	0.42	59087	44	***	287.67	480	0.64
GLC	µg/mg DW	60	53182	44	***	306.67	289	0.76	61996	43	***	295	557	0.61
TOTSUG	µg/mg DW	30	55417	44	*	185.33	703	0.44	54842	44	***	259	469	0.62
TOTSUG	µg/mg DW	60	55257	44	***	324	284	0.77	63918	43	***	306.67	566	0.62
SUC	µg/mg DW	30	609.17	44	***	4.01	1.8	0.87	621.64	44	***	2.74	5.91	0.58
SUC	µg/mg DW	60	190.18	44	***	1.06	1.16	0.73	192.98	43	***	0.9	1.78	0.60
ABA	pmol/mg DW	30	3356.6	44	***	22.17	9.8	0.87	10622.5	44	***	68.93	34.6	0.86
ABA	pmol/mg DW	60	4616.4	44	***	26.13	26.5	0.75	9792.2	43	***	56.77	57.4	0.75

Genotype-by-environment (G×E) interactions can cause important rank changes among genotypes evaluated in diverse environments, heritability assessment consistent to response to selection based on overall environment means can be compared with heritability established within subsets of local environment means to determine the optimal selection strategy and promising genotypes (Atlin et al., 2000).

While heritability calculated for a single, uniform (well controlled) environment is informative in ranking traits according to their H^2, a difficulty in plant breeding is to identify

genotypes with consistent performance across different environments representative of those expected in the target geographic region and year-to-year variability in the climate. Single-environment $H^2$is a function of genotypic variance relative to variance between replicate plants that are experiencing the same environment, which was tightly managed in the present investigation. To evaluate the consistency of genotype performance for the phenotyped traits across the two distinct watering environments, a combined model was used to analyze effects due to environment and genotype × environment interaction (G × E), as well as genotype and error (residual). Heritabilities in this combined model include G × E contribution to the phenotypic variance, and thus are more realistic in relation to the feasibility of using phenotypic traits as the basis for breeding cassava for adaptation to a wide range of environments. As shown in Table 3.2, heritabilities for the combined-environment model are generally lower than in the single-environment model (Table 3.1), as expected. Leaf starch and sugars generally had quite low H^2, except sugars at DAY 30. Heritability of soil water content, which measures the extent of water extraction by roots, was < 0.5, and H^2 of leaf retention was < 0.6 at both dates of sampling. Height of the retained leaf region at the top of the plants (HRL) had much higher heritability at DAY 30 than at DAY 60, probably because at DAY 30 leaf abscission was at incipient stages, and HRL is partly a function of plant height, which had a very high H^2. At DAY 60 when abscission was severe (Chapter 2) and leaf retention was presumably most important, H^2 was only 0.26. For canopy temperature, H^2 was lower in the combined model, but still relatively high (generally > 0.7). Despite the expected tight association of ABA with water stress, heritability in the combined model across the watering environments was high at DAY 30, though it was only 0.58 at DAY 60.

As shown in Table 3.1, for several biomass traits (AGB_{FW}, SR_{DW}, SR_{FW}, FR_{DW}, FR_{FW}, and PI), residual variance (σ^2_e) was much lower in the water stress than well-watered environment. For the combined analysis, this condition does not satisfy the requirement that errors are normally and independently distributed with common variance [$\varepsilon \sim NID(0, \sigma^2)$]. To address this problem, data for these traits were transformed by taking logs, and the resulting data are shown in Table 3.2. The combined-environment model had much lower H^2 for storage root water content (decreased to just 0.13) and somewhat lower H^2 for storage root dry and fresh weight. However, H^2 for aboveground biomass fresh weight (AGB_{FW}), fibrous root fresh and dry weight, and partitioning index were high (≥ 0.77) in the combined model.

The G × E interaction effect was partitioned into its two components, i) heterogeneity of genotypic variance among environments [$V(\sigma_{g(env)})$], and ii) lack of genetic correlation between environments [$L(r_{g(env)})$] (Table 3.2). For most traits the preponderance of the G × E variance was due to lack of genetic correlation between environments. In plant breeding, the lack of correlation among environments creates difficulty using multi-environment field trials for phenotyping and identification of lines with wide adaptation because it changes the relative ranking and extent of superiority among lines depending on the environment in which they are grown. The traits with very low $L(r_{g(env)})$ also had low heritability (HRL at DAY 60 and starch at DAY 30); however, there was a poor correlation between H^2 and $L(r_{g(env)})$. Among the traits with high H^2 and low $L(r_{g(env)})$ (the most advantageous combination from a plant breeding perspective) were plant height at DAY 60 and ABA at DAY 30. Thus, for most traits, G×E interactions lowered heritability due to the lack of genotypic correlation in relative value in the two environments. Nevertheless, given that the heritabilities were quite high in many traits, the

complication to selection due to lack of correlation between the genotype's performances among different environments is relatively minor in the current study.

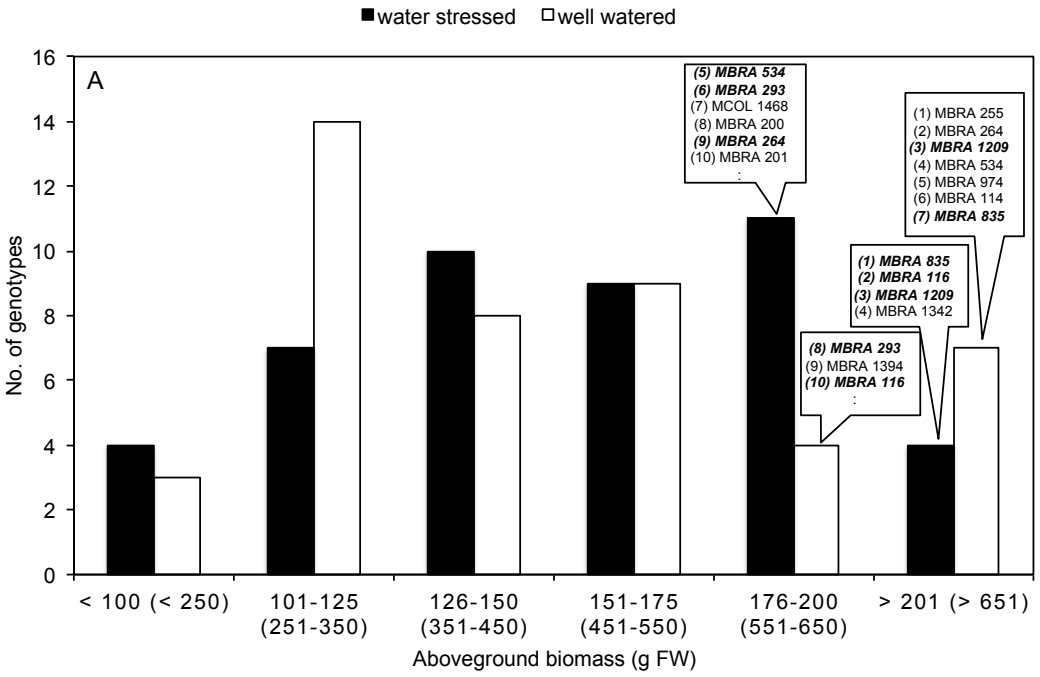
Table 3.2. Variance components for combined analysis across environments, and partitioning of G × E interaction into a component due to heterogeneity of genotypic variance ($V(s_{g(env)})$) and lack of genetic correlation among environments ($L(r_{g(env)})$).

Trait	Unit	Sampling Day	G×E analysis					
			σ^2_G	σ^2_{GE}	% $V(s_{g(env)})$	% $L(r_{g(env)})$	H^2	$r_{g(env)}$
AGB (log)	g FW	60	0.011	0.0026	28	72	0.81	0.85
SR (log)	g DW	60	0.035	0.0381	1	99	0.55	0.47
SR (log)	g FW	60	0.044	0.0201	0	100	0.73	0.69
SR_{wc}	g	60	0.00023	0.002	5	95	0.13	0.09
FR (log)	g FW	60	0.013	0.0042	26	74	0.77	0.81
FR (log)	g DW	60	0.023	0.0026	112	-12	0.82	1.01
#SR		60	0.389	0.1966	8	92	0.67	0.7
PI (log)		60	0.047	0.0186	0	100	0.77	0.72
PH	cm	30	139.47	4.57	1	99	0.97	0.97
PH	cm	60	190.3	14.42	58	42	0.94	0.97
HFS	cm	30	5.78	2.19	1	99	0.73	0.73
HFS	cm	60	37.57	42.31	64	36	0.62	0.71
HRL	cm	30	118.1	2.5	1	99	0.97	0.98
HRL	cm	60	16.91	84.16	74	26	0.26	0.44
LR	%	30	15.17	10.77	0	100	0.59	0.59
LR	%	60	14.54	22.9	0	100	0.47	0.39
CG	SPAD	30	16.45	3.33	0	100	0.85	0.83
CG	SPAD	60	16.45	6.85	3	97	0.76	0.71
T	°C	30	4.05	2.26	11	89	0.73	0.67
T	°C	60	4.3	3.51	4	96	0.64	0.56
*d*T	°C	30	8.09	3.48	8	92	0.78	0.72
*d*T	°C	60	6.63	3.31	13	87	0.77	0.7
$\theta_{0\text{-}5}$	$m^3 \times m^3$	30	0.00009	0.00005	62	38	0.49	0.8
$\theta_{0\text{-}5}$	$m^3 \times m^3$	60	0.00009	0.00013	15	85	0.4	0.45
$\theta_{20\text{-}25}$	$m^3 \times m^3$	30	0.00017	0.00033	43	57	0.4	0.48
$\theta_{20\text{-}25}$	$m^3 \times m^3$	60	0.00022	0.00054	60	40	0.35	0.51
STR	µg/mg DW	30	523.22	770.57	91	9	0.27	0.88
STR	µg/mg DW	60	456.27	-205.4	ns	ns	0.35	ns
GLC	µg/mg DW	30	272.17	-41.93	-17	117	0.78	1.22
GLC	µg/mg DW	60	12.27	303.51	0	100	0.05	-0.03
TOTSUG	µg/mg DW	30	258.96	-36.63	-8	108	0.77	1.18
TOTSUG	µg/mg DW	60	15.11	314.21	0	100	0.06	-0.02
SUC	µg/mg DW	30	2.26	1.12	5	95	0.65	0.68
SUC	µg/mg DW	60	0.48	0.5	1	99	0.49	0.49
ABA	pmol/mg DW	30	35.66	9.88	65	35	0.8	0.91
ABA	pmol/mg DW	60	22.3	18.81	16	84	0.58	0.59

3.3.2 Genotype frequencies and performance "per se"

In order to further examine the relative rankings of genotype performance under stress and well-watered conditions, storage root fresh weight and yield component frequency histograms and ranking tables were produced and are presented in Figure 3.1 and Table 3.3. In our selection of best yielding genotypes under stress (RANKS 1 to 10; Figure 3.1 B; Table 3.3 A) for storage root fresh weight (SR_{FW}), we found several cassava genotypes that also placed high in well-watered conditions. For example, genotypes MBRA 165 and CM 3306-9 ranked first and second for SR_{FW} under stress and fifth and second in well-watered conditions, respectively. In addition, these two genotypes ranked first and second for partitioning index (PI) in both environments (Tables 3.3 A and B). The 10 best-ranked genotypes for storage root fresh weight yielded a mean of 28.9 g FW in water-stressed conditions, 2-fold higher than the population mean. Regarding SR_{FW} in well-watered conditions, the 10 best-ranked genotypes were 69% higher when compared to the population mean. Frequency histograms for fibrous root fresh weight (FR_{FW}), number of storage roots (#SR), and partitioning index (PI) for all genotypes under stress and well-watered conditions were similar and grouped several identical genotypes at the right-hand tail of the distributions, indicating comparable performances in both environments (Figures 3.1 C-E).

Figure 3.1 Frequency histograms of all 45 cassava genotypes for above ground biomass fresh weight (A), storage root fresh weight (B), fibrous root fresh weight (C), number of storage roots (D), and partitioning index (E) under well-watered and water-stressed conditions measured at DAY 60. Horizontal axis values in parentheses and without parentheses are for well-watered and water-stressed conditions, respectively. Genotypes in square pop outs are ranked depending on performance by trait. Corresponding genotypes within environments indicated in **bold** and *italics*.


■water stressed □well watered
A
No. of genotypes
0
2
4
6
8
10
12
14
16
(5) MBRA 534
(6) MBRA 293
(7) MCOL 1468
(8) MBRA 200
(9) MBRA 264
(10) MBRA 201
:
(1) MBRA 255
(2) MBRA 264
(3) MBRA 1209
(4) MBRA 534
(5) MBRA 974
(6) MBRA 114
(7) MBRA 835
(1) MBRA 835
(2) MBRA 116
(3) MBRA 1209
(4) MBRA 1342
(8) MBRA 293
(9) MBRA 1394
(10) MBRA 116
:
< 100 (< 250)
101-125 (251-350)
126-150 (351-450)
151-175 (451-550)
176-200 (551-650)
> 201 (> 651)
Aboveground biomass (g FW)

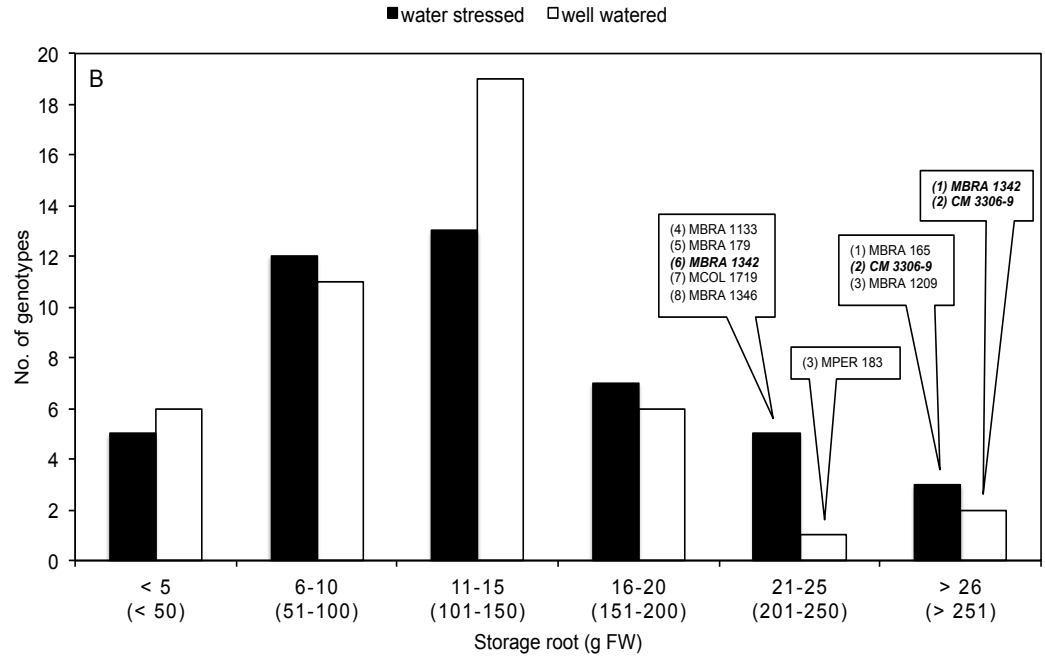
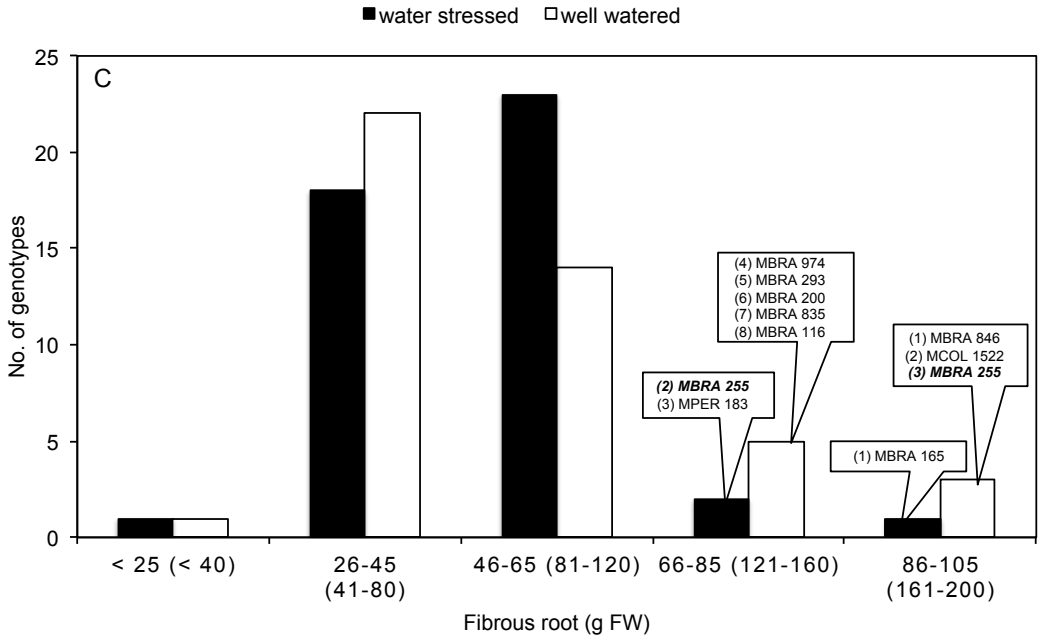

■water stressed □well watered
B
No. of genotypes
0
2
4
6
8
10
12
14
16
18
20
(4) MBRA 1133
(5) MBRA 179
(6) MBRA 1342
(7) MCOL 1719
(8) MBRA 1346
(3) MPER 183
(1) MBRA 165
(2) CM 3306-9
(3) MBRA 1209
(1) MBRA 1342
(2) CM 3306-9
< 5 (< 50)
6-10 (51-100)
11-15 (101-150)
16-20 (151-200)
21-25 (201-250)
> 26 (> 251)
Storage root (g FW)

Figure 3.1 (Continued)



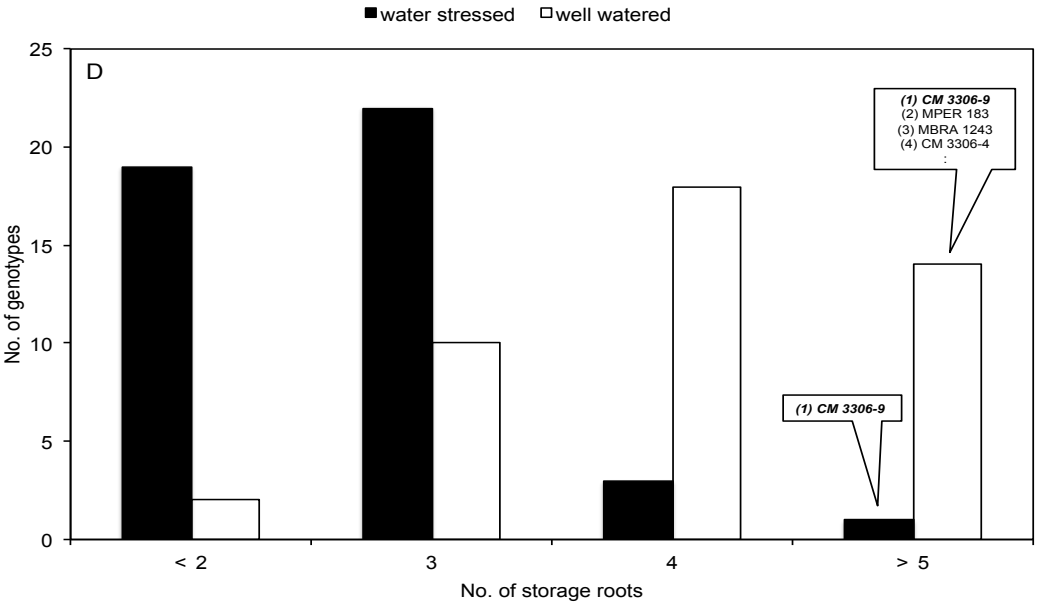


Figure 3.1 (Continued)

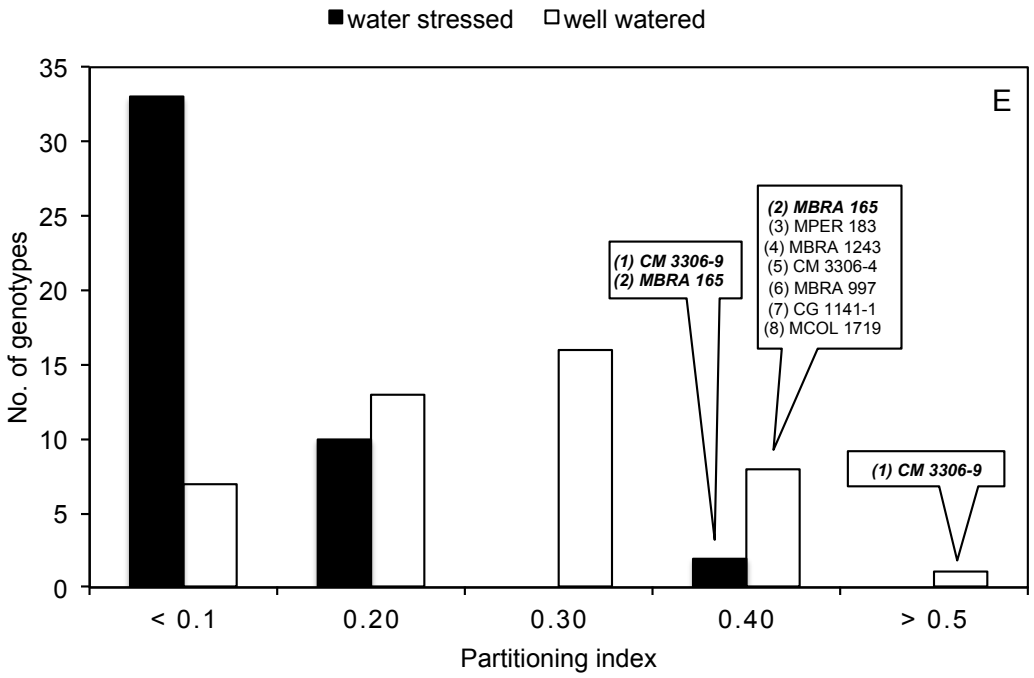


Table 3.3 Storage root fresh weight; yield components, and ranks under well-watered conditions of the 10 highest-yielding genotypes under stress (A) and of the 10 highest-yielding genotypes under well-watered conditions (B). $\bar{X}$ = sample mean, μ = population mean.

Table 3.3A

Genotype	SR_{FW}		RANK		AGB_{FW}		RANK		FR_{FW}		RANK		#SR		RANK		PI		RANK	
	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW
MBRA 165	69.6	186.7	1	5	138.4	344.4	28	30	89.2	72.8	1	27	1.2	4.2	5	14	0.3	0.4	2	2
CM 3306-9	47.0	252.4	2	2	98.2	246.8	43	43	39.2	51.4	33	41	4.2	7.0	45	1	0.3	0.5	1	1
MBRA 1209	26.2	168.0	3	8	203.4	675.8	3	3	55.4	77.6	12	24	2.2	4.2	21	12	0.1	0.2	9	23
MBRA 1133	23.3	94.4	4	31	127.0	341.8	33	31	31.2	54.4	43	40	2.5	4.0	30	20	0.1	0.2	6	24
MBRA 179	22.8	141.2	5	11	176.6	417.4	14	23	31.2	42.4	42	44	2.4	3.6	28	25	0.1	0.2	8	18
MBRA 1342	20.8	272.8	6	1	202.0	554.2	4	11	59.8	80.4	8	22	2.2	4.2	20	11	0.1	0.3	15	10
MCOL 1719	20.6	128.4	7	17	97.8	287.6	44	36	22.4	44.8	45	43	3.0	4.6	41	7	0.2	0.3	3	8
MBRA 1346	20.3	125.4	8	19	102.8	300.0	41	33	35.2	58.0	37	36	3.0	3.4	40	29	0.2	0.3	4	14
MBRA 997	20.0	174.4	9	6	163.6	371.2	19	27	40.8	76.4	31	25	3.2	3.6	42	24	0.1	0.3	10	6
CG 1141-1	19.0	129.6	10	16	109.2	265.6	39	39	46.6	87.4	23	20	3.0	4.2	39	10	0.1	0.3	5	7
X	28.9	167.3			141.9	380.5			45.1	64.6			2.7	4.3			0.2	0.3		
μ	14.4	116.5			152.0	436.2			48.2	88.7			2.3	3.7			0.1	0.2		
$Tukey_{HSD(0.05)}$	20.6	158.9			76.5	395.0			35.0	83.5			2.0	2.8			0.1	0.2		

Table 3.3 (Continued)

Table 3.3B

Genotype	SR_{FW}		RANK		AGB_{FW}		RANK		FR_{FW}		RANK		#SR		RANK		PI		RANK	
	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS
MBRA 1342	272.8	20.8	1	6	554.2	202.0	11	4	80.4	59.8	22	8	4.2	2.2	11	20	0.3	0.1	10	15
CM 3306-9	252.4	47.0	2	2	246.8	98.2	43	43	51.4	39.2	41	33	7.0	4.2	1	45	0.5	0.3	1	1
MPER 183	245.8	12.4	3	22	434.2	162.8	22	20	97.8	67.0	16	3	4.0	3.8	19	43	0.4	0.1	3	26
MBRA 264	196.0	14.4	4	18	694.0	183.0	2	9	80.0	50.8	23	19	3.6	2.0	23	15	0.2	0.1	22	23
MBRA 165	186.7	69.6	5	1	344.4	138.4	30	28	72.8	89.2	27	1	4.2	1.2	14	5	0.4	0.3	2	2
MBRA 997	174.4	20.0	6	9	371.2	163.6	27	19	76.4	40.8	25	31	3.6	3.2	24	42	0.3	0.1	6	10
MCOL 1468	172.2	18.4	7	11	472.8	185.8	19	7	80.6	46.0	21	25	3.4	2.0	27	16	0.3	0.1	16	16
MBRA 1209	168.0	26.2	8	3	675.8	203.4	3	3	77.6	55.4	24	12	4.2	2.2	12	21	0.2	0.1	23	9
MBRA 346	156.0	12.3	9	23	507.2	149.6	14	25	64.8	41.2	32	30	4.6	2.0	5	17	0.3	0.1	17	19
MVEN 25	148.2	9.2	10	31	322.4	153.6	32	24	64.2	45.8	34	26	4.6	1.4	6	7	0.3	0.1	9	30
X	197.2	25.0			462.3	164.0			74.6	53.5			4.3	2.4			0.3	0.1		
μ	116.5	14.4			436.2	152.0			88.7	48.2			3.7	2.3			0.2	0.1		
$Tukey_{HSD(0.05)}$	158.9	20.6			395.0	76.5			83.5	35.0			2.8	2.0			0.2	0.1		

3.4 Discussion

In this study, 45 contrasting cassava genotypes were compared for their performance in two distinctly different watering environments. Cassava is grown in a wide range of environments. Moreover, given cassava's relatively long growth cycle, it is common that its growth spans at least one dry season, between one or more wet seasons. Unpredictable year-to-year variation in the timing and severity of the dry seasons creates a situation where the best lines are those that can tolerate a wide range of environments. For example, in the environment in Colombia where the study was performed, annual rainfall is between 800 and 1500 mm, however, the region presents a bimodal rainfall distribution with two dry seasons with occasional severe drought episodes. Thus it is hypothesized that the most suitable genotypes should maintain relatively moderate to high yields in both well watered and water stressed environments.

To address the need for cultivars with superior plant performance in environments spanning a wide range of water availabilities, the present investigation examined the relative performance of 45 genotypes representing lines that are being used as germplasm for cassava breeding. In plant breeding, the average predicted response to one cycle of phenotypic selection over all environments (ΔG) is:

[9] $$\Delta G = ih^2\sigma_p$$

where $\boldsymbol{i}$ is the standardized selection intensity, $\boldsymbol{h^2}$ is the narrow-sense heritability and, σ_p is the phenotypic standard deviation. This study showed that for many of the traits which were examined, broad sense heritability and phenotypic standard deviation were sufficiently high to predict that response to selection would be successful. This study also examined the components of G × E variance, which detract from heritability, and showed that for most traits, the lack of correlation between genotypic performances in the watering environments was responsible for G × E effects. Although this component of G × E causes more complication in breeding than the component of heterogeneity of genetic variance between environments, this study further showed that within the panel of 45 genotypes, it was possible to identify some with high-ranking performance in both watering environments.

3.4.1 Variance components and heritability performance

Since heritabilities can differ between traits within a population, genetic correlations estimates among traits coupled with heritability estimates of different traits can be utilized to identify indirect selection strategies that may be more effective than direct selection strategies (Banziger et al., 2002; Diz and Schank, 1995; Rebetzke et al., 2002).

Effects on variance components and heritability consisting of genotype means conducted in a single year are presented in Table 3.1. As stated above, the genotypic and residual variance components were approximately equal in magnitude in both environments, indicating that several traits, including yield and yield components, were not subject to high genotype × environment interactions. In our study, residual variance components for storage root fresh weight under stress were lower when compared to controls and r_G between SR_{FW} and PI under non-stress and stress conditions were high and approached 1.0 (Appendix, Chapter 2). As a result, heritability for SR_{FW} in a single year was 0.90 under stress and 0.77 for controls.

Similarly, several of the morpho-physiological traits had higher H^2estimates than $SR_{FW;}$ however, r_G between these traits and SR_{FW} under stress were not close to 1.0, except for PI.

In rice, several authors have suggested that the use of secondary traits was predicted to be less effective in improving yield under stress than selecting for yield itself (Atlin and Lafitte, 2002). The study further indicated that indirect selection for grain yield under lowland non-stress will be less efficient as compared to direct selection under lowland reproductive stage drought stress and confirmed the similar finding reported earlier under upland stress (Venuprasad et al., 2007).

Kawano (2003) reviewed the relationship between heritability and correlation measures in cassava breeding and concluded that partitioning index has consistently high heritability at different evaluation stages, while biomass and yield presented low heritability. In addition, indirect selection for yield through partitioning index is more effective than direct selection by yield itself, especially in the early evaluation stages of cassava breeding.

In the current study, both genotypic and residual variance components were of similar magnitude for all morpho-physiological traits. However, storage root fresh weight and yield components presented dissimilar variance component values in magnitude in both stressed and controlled conditions. Regarding nonstructural carbohydrates and ABA, genotypic variance components were similar between environments except for leaf starch at both sampling dates. Residual variance components were also exceptionally high for leaf starch in both environments. Estimated heritabilities, although high in both environments for the majority of traits assessed, were not higher than for SR_{FW}.

Several traits such as plant height (PH), leaf canopy temperature (T), difference in external temperature (dT), leaf chlorophyll greenness (CG), and partitioning index (PI) under

stress presented predicted heritabilities that exceeded SR_{FW}. Hence, these measured traits in addition to being important for explaining physiological adaptations to drought tolerance and to an extent yield under stress, were measured accurately and on a high throughput basis. Thus, trait estimates indicate that, in breeding for drought tolerance, selection for them coupled with yield can equal direct selection for yield under stress. Therefore, for this study, there is evidence that utilizing indirect selection of secondary traits for yield under stress is a useful approach. The above mentioned traits exhibited a higher level of heritability under drought stress than under control conditions. This demonstrates that the drought screening protocol, which ensured that terminal water stress was imposed for the early stages of developmental growth, is highly repeatable. Thus, these traits, as well as early yield under stress, are potential targets for selection. None of the other secondary morpho-physiological parameters, including NSC accumulation or leaf retention had a predicted H^2 that exceeded that early tuber yield under stress. There was no evidence that genetic control for the other secondary traits was simpler than that of early yield, or that, with the exception of plant height, leaf canopy temperature, and leaf chlorophyll greenness, they could be measured with greater repeatability. They are therefore unlikely to be useful criteria for indirect selection for yield under drought stress. This situation might change if technical improvements result in more precise and repeatable measurement.

3.4.2 Genotype frequency and ranking performance

The concept of genotype ranking based on trait overall performance provides the breeder with an opportunity to identify the highest yielding entries or genotypes with a certain combination of highly desirable traits. Individual tests are performed and the best genotypes scored and continued through a series of additional tests. In our study, for example two

genotypes (CM 3306-9 and MBRA 165) consistently ranked high for SR_{FW} and partitioning index under stress as well as in control conditions (Figure 3.1 and Table 3.9). The cassava "hybrid family" known as "CM 3306" has produced excellent progeny tested for yield throughout several agroecological zones in Colombia (Ceballos et al., 2002). More recently, the clones CM 3306-4, also known as *ICA-Negrita* (also used in this study) and CM 3306-19 were released as high yielding clones for fresh market consumption in Colombia. In addition, clone CM 3306-4 (derived from the cross between COL 22 × CM 523-7) gave exceptional yield in drought stressed areas in the Northern coast of Colombia, more specifically in the La Guajira peninsula (11°51′0″N, 72°2′0″W; mean annual high temperature = 32.7°C and mean annual precipitation = 553 mm) (Ceballos et al., 2002). The land race MBRA 165 originated in northeastern Brazil, which is a semi-arid region characterized by desert-like vegetation thus giving us valuable information on its tolerance to water stress. These genotypes as well as several others need to be given special attention by breeders, as they may possess the desired combination of performance-based characteristics that are required for water stressed environment.

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CHAPTER 4:

Growth, carbohydrate and abscisic acid dynamics of contrasting cassava genotypes during water deficit stress

4.1 Introduction

Cassava (*Manihot esculenta* Crantz) is a storage root crop native to the neotropics and is of great economic and dietary importance in rural areas of the tropics (Hershey, 1984; Rogers and Fleming, 1973). In the majority of the tropics, it is sown and harvested by small holder farmers on marginal soil without artificial amendments or controlled irrigation (Cock et al., 1985). It is grown mainly for its starchy tuberous roots and is a key staple food for countless farmers in the tropics (Best and Henry, 1994). In addition, cassava foliage has excellent nutritional quality as a source of protein, vitamins, and minerals for animal and human consumption (Ceballos et al., 2004). Cassava grows reasonably well in low fertility soils and under water deprivation, making it an important staple crop on poverty-stricken marginal lands. Nevertheless, though cassava can endure several months of water stress during its seasonal developmental cycle, water stress still reduces its net biomass production greatly below its maximum yield potential (Calatayud et al., 2000; Calatayud et al., 2002; Connor and Cock, 1981; Connor et al., 1981; El-Sharkawy, 1993).

According to FAO statistics (FAOSTAT, 2011), worldwide production of cassava roots has nearly doubled in the last thirty years, reaching approximately 213 million tons fresh mass in 2005. Africa currently produces more than 50% of global production with 118 million tons. This constitutes an increase of approximately 70% compared with the 70 million tons produced in

1990. In addition, cassava production is expected to expand in Asia, especially following the annual planting survey in Thailand, which pointed to a 12% rise in production in 2007 to 25.3 million tons. There has also been an important surge in cassava production for Brazil because of the continuance of strong government support for the country's cassava sector producing approximately 26.7 million tons in 2008 (FAOSTAT, 2011). Much of this production was used for chips, pellets, and starch for the export markets as well as for human consumption and industrial raw material (i.e., for bio-ethanol production).

Thus, given the expanding supplies and demands worldwide, genotypes with high and consistent yields and with tolerance to biotic and abiotic stresses are needed. In this regard, selection for valuable morphological, structural, biochemical, and physiological traits that enhance yield and stress tolerance has the potential for raising agricultural productivity (El-Sharkawy, 2005; Richards, 2000). Nevertheless, cassava has received relatively little research attention compared to other crops grown in the tropics such as maize, rice, sorghum, potatoes, and beans. Thus, further research on cassava is justified, particularly as it relates to the primary abiotic environmental constraint: drought.

The two main components of drought resistance in plants are drought avoidance and drought tolerance (Blum, 2005). Cassava has elements of both. Regarding drought avoidance, cassava develops an extensive root system (Duque, 2007; El-Sharkawy et al., 1992a; Pardales and Esquibel, 1996; Pardales and Yamauchi, 2003; Tscherning et al., 1995) and reduces its water use by reducing leaf growth rate and by leaf abscission (Alves and Setter, 2000; Alves and Setter, 2004; Duque and Setter, 2005; El-Sharkawy et al., 1992a; El-Sharkawy et al., 1992b; Ike, 1982; Ike and Thurtell, 1981a; Ike and Thurtell, 1981b; Ike and Thurtell, 1981c; Itani et al., 1999; Lenis et al., 2006). It also limits water use by stomatal closure at modest water deficits that

maintains high leaf relative water content (RWC) (Indira and Ramanujam, 1984). Furthermore, another category of plant response to drought that is important in crops is altered partitioning and utilization of carbohydrates, which are limited during stress because of decreased photosynthesis. In cassava, a contributor to drought tolerance involves its ability to accumulate substantial carbohydrate reserves in its stem, which are slowly remobilized during stress episodes (Duque and Setter, 2005). Another factor is the extent to which a genotype is able to maintain growth of the storage root during stress. In a few studies it appears that genotypes well adapted to water stress initiate storage root growth early during development and maintain partitioning of limited carbohydrates toward the storage root during the stress (Okogbenin and Fregene, 2002; Okogbenin and Fregene, 2003; Okogbenin et al., 2003).

The goal of the current study is to investigate a set of cassava genotypes that differ in drought tolerance to determine their early response to water deficit as a way to evaluate which traits are associated with genotypic differences in drought tolerance. This information will assist cassava breeders in their efforts to identify, understand, and then put into practice manageable screening methods in order to develop elite cassava germplasm with superior drought tolerance. The specific objective of this study was to determine the influence of early water-deficit stress on the temporal dynamics of above ground growth, physiological traits related to water deficit (ABA and carbohydrate reserves), and biomass partitioning at an early stage of storage root growth in several contrasting cassava genotypes.

4.2 Materials and Methods

4.2.1 Plant Material

To determine the effects of artificially induced water stress without re-watering on several morpho-physiological traits, a screen house experiment was set up, which included 15 contrasting cassava genotypes obtained from the International Center for Tropical Agriculture (CIAT) cassava core germplasm collection located in Palmira, Colombia, South America. Germplasm was carefully selected by HernanCeballos, Team Leader of cassava breeding at CIAT, to include a range of drought tolerant and drought susceptible landraces and improved lines and was classified as drought susceptible or drought tolerant based on more than 20 years of yield trials in different agro-ecological areas throughout Colombia and Brazil. The genotypes varied in genetic background and have been tested for different climates (Table 4.1).

Table 4.1 List of the 15 cassava genotypes used for well watered and water stressed experiments in this study. The code "*M*" denotes *Manihot*. *CM* and *CG* codes identify genotypes derived from CIAT's cassava breeding project. The remaining genotypes are from the germplasm bank collection. The column labeled as "Type" denotes drought tolerant (TOL) or drought susceptible genotypes (SUS).

Genotype	Common name	Origen	Type	Biological status	Selection
MBRA 1133	Veada 1	Brazil	TOL	Landrace	EMBRAPA/CIAT
MBRA 1243	Sapa R-16	Brazil	TOL	Landrace	CIAT
MBRA 1435	Raimunda	Brazil	TOL	Landrace	CIAT
MBRA 255	EnganaLadrao	Brazil	TOL	Landrace	EMBRAPA/CIAT
MBRA 293	Amansa Burro	Brazil	TOL	Landrace	EMBRAPA/CIAT
CG 1141-1	ICA-Costeña	Colombia	TOL	Improved line	CIAT
CM 2772-3	N/A	Colombia	SUS	Improved line	CIAT
CM 3306-4	ICA-Negrita	Colombia	TOL	Improved line	CIAT
CM 4919-1	Corpoica Veronica	Colombia	TOL	Improved line	CIAT
CM 507-37	N/A	Colombia	TOL	Improved line	CIAT
MCOL 1468	Mantiqueira	Brazil	SUS	Landrace	EMBRAPA/CIAT
MCOL 1684	Charay	Colombia	SUS	Landrace	EMBRAPA/CIAT
MPER 183	Eeat 1	Peru	SUS	Landrace	CIAT
MTAI 8	CMR 246343 (Ryg.60)	Thailand	TOL	Improved line	EMBRAPA/CIAT
MVEN 25	QuerepaAmarga	Venezuela	SUS	Landrace	CIAT

4.2.2 Screen House Management

Approximately 50 stem cuttings (25-30 cm long) of each genotype were disinfected and sown in plastic bags containing 50 kg sterilized mineral soil: coarse sand (2:1) (15 genotypes × 50 plants = 750 total plants). Next, plants were placed inside a screen house [corrugated transparent polycarbonate plastic roof, and side-walls of anti-insect screen, Polyethylene monofilament, 266 × 818 micron mesh hole opening size, used to avoid the entrance of the virus vector Whitefly (*Trialeurodes vaporariorum*)] and received manual irrigation. At 60 days after planting (DAP), the 50 plants of each genotype were randomly assigned to 10 discrete plots. Plant height (PH) at DAY 0 ranged between 150 and 170 cm for all genotypes tested. One plot of each genotype was then assigned to each of ten blocks and these blocks were randomly assigned to either the well watered (WW) or water stressed (WS) treatments (five blocks to each treatment), as described below. Thus, each block contained a complete set of plots representing all genotypes and watering treatments, and each plot contained five plants to permit five dates of sampling as described in the next section. Blocks were arranged evenly within the screen house such that all plants within the same block experienced about the same screen house lighting, temperature, ventilation, and other environmental conditions. Within each plot, plants were evenly spaced in a grid layout (0.8 m × 0.8 m) measured from the center of each stem. The distance between plots in all directions was approximately 1.5 m. At this stage, referred to as DAY 0, two water treatments were imposed: (i) control (plants were irrigated every other day until drainage occurred) and (ii) water stress (irrigation was withheld and soil was allowed to dry for the duration of the experiment). All plants were maintained inside the screen house for the duration of the experiment.

4.2.3 Growth Parameters

Plants within a plot were randomly assigned to five sampling dates to assess development stage effects in response to WW and WS treatments. The first sampling date was at 60 DAP (referred as to DAY 0), at which point irrigation in the WS treatment was stopped. The second sampling date was 15 days after DAY 0 (referred as to DAY 15) and in successive manner, DAY 30, DAY 45, and finalizing at DAY 60. At each sampling date, the following morpho-physiological traits were recorded (described in detail in later sections): plant height (PH); leaf retention (LR); volumetric soil water content at 0-5 and 20-25 cm depth ($\theta_{0\text{-}5}$ and $\theta_{20\text{-}25}$); aboveground fresh biomass (AGB_{FW}); storage root fresh weight (SR_{FW}); fresh weight partitioning index (PI); leaf, stem and root abscisic acid (ABA_L, ABA_S, ABA_R); leaf, stem and root non-structural carbohydrates (NSC_L, NSC_S, NSC_R); and leaf relative water content (RWC).

4.2.3.1 Plant height

Plant height (PH) was measured as the distance from the soil surface at the base of the main stem to the uppermost fully expanded leaf.

4.2.3.2 Leaf Retention

Increased longevity of leaves, or improved leaf retention, has been suggested as a possible means to increase productivity of cassava in water-stressed environments (Lenis et al., 2005). In cassava, leaf abscission advances in a highly predictable pattern starting at the lowest stem node and advancing upward, with retained leaves in the apical section of the stem and branches. Leaf retention per genotype was scored on a percentage basis measuring the total plant height from the soil surface compared to the length from the first intact leaf-petiole to the

uppermost apical meristem on the main stem (height of the stem containing retained leaves, HRL). From these values leaf retention (LR) was calculated from the following expression:

$$LR(\%) = \frac{HRL}{PH} \times 100$$

This procedure was adopted in order to ensure fair comparison among all genotypes.

4.2.3.3 Soil water content

Volumetric soil water content (θ, $m^3 \times m^3$) was measured in the first 0-5 cm and 20-25 cm soil layers on each plant using a ThetaProbe Soil Moisture Sensor (model ML2x; Delta-T Devices, UK). A set of three-pronged waveguide rods made of stainless steel, 20 cm long and 3.0 mm in diameter, was inserted horizontally in each soil layer and allowed to equilibrate. A total of two measurements per soil layer were taken and averaged.

4.2.3.4 Yield components

At each sampling date, plant biomass and its components were measured including aboveground biomass fresh weight (AGB_{FW}), storage root fresh weight (SR_{FW}), and partitioning index (PI). A plant from each WW and WS plot was harvested at DAY 0, 15, 30, 45, and 60. Storage roots were defined as roots >5 mm diameter (Ø). Partitioning index (PI) was measured as the ratio between storage root fresh weight and total biomass expressed in the following equation:

$$PI = \frac{SR_{FW}}{AGB_{FW} + SR_{FW}} \times 100$$

4.2.3.5 Non-structural carbohydrates and abscisic acid

At each sampling date (DAY 0, 15, 30, 45, and 60) a total of three tissue samples were collected from each plot. For mature and immature leaves, three leaf disks (diam. = 0.635 cm) were sampled from three mature fully expanded leaves and another three from the three uppermost folded immature (expanding) leaves to form a composite sample. Subsequently, cylindrical stem plug samples from the middle third of the shoot system were obtained utilizing a 3 mm ∅ cork borer. Three root tips were sampled by cutting about 1 cm of small healthy portions from the new root growth to form a composite sample per plant. All plant tissues were sampled at DAY 0, 15, 30, 45, and 60 between 1100 and 1400 hours. Sampled tissue was immediately immersed in 300 µL of ice-chilled (0ºC) 80% methanol. All leaf measurements were expressed on an area basis; stem and root measurements were expressed on a dry weight basis. Sucrose, glucose and, starch were measured on all plant tissue using the peroxidase/glucose oxidase (PGO) method as described by (Ober et al., 1991) and (Setter et al., 2001). The PGO method is based on the Trinder reaction, where glucose reacts with O_2 (catalyzed by glucose oxidase) to form gluconic acid and H_2O_2. Catalyzed by peroxidase, the H_2O_2 immediately reacts with *p*-hydroxybenzoic acid and 4-amino-antipyrine to create a bright pink dye complex (Trinder, 1969). Crude extracts were used for plant tissue samples analyzed for sucrose and glucose content. Dried sample extracts were re-dissolved in a known volume of 0.01% azide water, and an aliquot was transferred to 96-well plates containing 50 µl autoclaved water. To analyze glucose content, 150 µl of PGO solution (peroxidase and glucose oxidase enzymes in buffer solution containing 100 mM KH_2PO_4-NaOH (pH 7.0), 10 mM para-hydroxybenzoic acid, 0.001 mM 4-aminoantipyrine, 0.1% (w/w) bovine serum albumin, and 0.01% sodium azide) was added to each well containing leaf, stem, and root samples. After full

color development at room temperature, the plates were read on a Packard SpectraCount model 750 photometer (490 nm wavelength setting). To quantify total sugars (including sucrose) content, an invertase solution (292 U/mg, 10mg/mL H_20) was added to the samples, and reaction was allowed to run until full color development of sucrose standards before reading on the photometer (490 nm). Standards made from dilutions of glucose (0 to 32 ug/well) and sucrose (25 ug/well) solutions were used to calibrate the assay.

After all free sugars were extracted, starch content was also determined. After samples were dried overnight, each sample was rediluted in 200 µL azide water, covered, and incubated at 80°C to gelatinize starch. After two hours, samples were cooled and 200 µL enzyme solution (250 mM acetate buffer at pH 4.5, 74 U/mg amyloglucosidase, 20 U/mg α-amylase, 0.1% w/v sodium azide, and 0.1% BSA) were added to hydrolyze starch into glucose. The reaction was incubated for two days on a rotary shaker at 37°C. Samples were then stored in 5°C. The PGO method was then used to determine the amount of glucose cleaved from starch.

Prior to hormone analysis, leaf tissue was first separated into fractions based on hydrophobicity using reverse phase C_{18} chromatography, as described by Setter and Parra (2010), modified from (Ober et al., 1991) and (Setter et al., 2001). The method involves the following steps, Supelco columns (DSC-18 SPE-96) with 25 mg of C_{18} packing material were used in a 96-well vacuum apparatus. Columns were washed with 95% (v/v) ethanol/water and 30% (v/v) methanol/water prior to use. Extracts from samples stored in 80% methanol were transferred to a 96-well plate, dried in a forced-air incubator at 45°C, then redissolved in 100 µL 30% methanol and 1% v/v glacial acetic acid with 20 µL 0.04% bromecresol green added as a chromatograph tracer. Samples were loaded onto the columns with 120 µL 30% methanol, and pulled through by vacuum. Columns were then washed with 200 µL 30% methanol to remove

any remaining hydrophilic compounds. Abscisic acid was eluted from the columns using 200 µL 65% methanol with 1% acetic acid, followed by a 200 µL 95% ethanol to remove any lingering compounds. NH_4OH was added to neutralize the acetic acid. Plates were read on a spectrophotometer (Packard SpectraCount model 750) using a 590 nm wavelength to measure bromecresol green. Abscisic acid (ABA) levels were determined using an enzyme-linked immunosorbant assay (ELISA) as described in Setter et al. (2001). The 65% methanol fractions from reverse phase C_{18} chromatography were used for ABA quantification. After drying, samples were redissolved in 100 µL azide water (0.01%). Next, 96-well plates were coated overnight with a BSA conjugate solution (ABA-BSA for abscisic acid) containing 1.4 µg BSA conjugates per plate and 50mM $NaHCO_3$ at a pH of 9.6. Plates were then washed four times with a TBS (10mM tris-hydroxymethylaminomethane-HCl, pH of 7.5, 1 mM $MgCl_2$, 100mM NaCl) and 0.1% Tween-20 solution (TBST). Dried samples were redissolved in 200 µL of water (containing 0.01% azide) and 20 µL was dispensed into 90 µL of MBSA (50mM MOPS-NaOH, pH 7.5, 1 mM $MgCl_2$, 100 mM NaCl) and 100 µL of primary antibody solution (100 µL MBSA containing 1 µg of anti-ABA) (Setter *et al*., 2001). A calibration curve was generated using a serial dilution of ABA standards ranging from 0.002 to 5 pmol/well. After incubating overnight at 5°C, the plates were again washed four times with a TBST solution. Secondary antibody solution (200 µL containing 10 nL of anti-mouse IgG-alkaline phosphatase (reporter enzyme) conjugate in MBSA) was added to each well. Reaction was run overnight at 5°C. After washing four times with a TBST solution, 200 µL PNPP (0.2 mg *p*-nitrophenyl phosphate in 0.9 M diethanolamine and 3 mM $MgCl_2$ at pH 9.8) substrate solution was added and the reaction incubated for 60 min at room temperature before reading absorbance and 405 nm with spectrophotometer (Packard SpectraCount model 750).

4.2.3.6 Relative water content (RWC)

RWC describes a convenient indicator of the water balance state of a plant, fundamentally because it expresses the quantity of water in a tissue relative to the absolute quantity of water which the plant would need to achieve complete saturation and can be used to assess leaf water content in water stress scenarios (Gonzalez and Gonzalez-Vilar, 2003). Measurements of leaf water status were performed between 1100 and 1400 hours on each of the sampling dates. A composite sample of 3 leaf discs (diam. = 1.905 cm) was sampled from three mature fully expanded leaves. Leaf RWC was determined with the following equation (Smart and Bingham, 1974):

$$RWC(\%) = \frac{FW - DW}{TW - DW} \times 100$$

Leaf fresh weight (FW) was determined immediately after sampling, whereas turgid weight (TW) was determined by soaking the composite leaf samples in distilled water in test tubes for up to 12 hours at 20°C. After soaking, leaf samples were quickly and carefully blotted dry with Kimwipe (Kimberly-Clark, Roswell, GA USA) tissue paper in preparation for determining turgid weight. Dry weight (DW) was assessed after oven drying leaf samples at 60°C for 48 hours.

4.2.4. Statistical analysis

All data were subjected to analysis of variance (ANOVA) using a JMP 9.0 statistical package (SAS Institute, Inc. U.S.A.). The model contained the following factors: genotype drought susceptibility/tolerance category type (G), watering treatment (T), G x T interaction,

sampling date (S), S x T, S x G, and residual. The significance of factor effects was determined using the F-test. Results are means ± sem or ± pooled sem when indicated. Honestly significant difference (HSD) between resulting means were estimated by Tukey's test ($p< 0.05$).

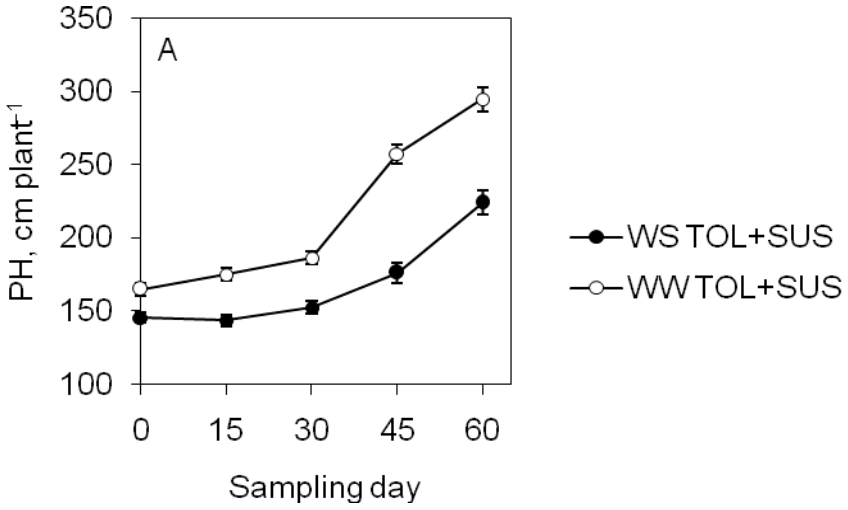
4.3 Results

4.3.1 Growth Patterns of morpho-physiological traits

In general, water stress halted growth and development of several morpho-physiological traits when compared to well-watered controls. Plant height (PH) was less in water stress than control plants from DAY 15 (Fig. 4.1A) and remained low until the end of the experiment, at which point PH of WS plants was ~25% less than controls. Water stress reduced aboveground biomass fresh weight (AGB_{FW}) and total root fresh weight ($TOTALROOT_{FW}= SR_{FW} + FR_{FW}$) measured after DAY 15 and they remained low until DAY 60 (Fig. 4.1C and D). At Day 60, plants subjected to water stress had a reduction of ~52% in storage root fresh weight (SR_{FW}) and ~58% in fibrous root fresh weight (FR_{FW}) when compared to controls (Fig. 4.1F). The number of storage roots decreased from 2.3 ± 0.2 (mean ± SEM) in well-watered plants to 1.2 ± 0.2 in water stressed conditions (Fig. 4.1F). To study the effects of water deficit on leaf abscission, leaf retention (LR) was assessed as a proportion of plant height. Water stress induced leaf abscission whereas leaf abscission was substantially less in control plants (Fig. 4.1E). However, some leaf abscission was observed in well-watered controls (~20%). Interestingly, plants subjected to water stress had a minimum percent of leaf retention at Day 30, then increased leaf retention until the end of the experiment due to regrowth of new leaves at the apical meristem level. Although slowed by WS, plant height increased and new leaf formation (personal observation)

continued from DAY 30 to DAY 60 (Figure 4.1A), and the measured value for LR was a function of both leaf abscission and new leaf formation.

Figure 4.1 Growth parameters of (A) plant height (PH), (B) leaf relative water content (RWC), (C) aboveground biomass fresh weight (AGB_{FW}), (D) total root fresh weight ($TOTALROOT_{FW}$), (E) leaf retention (LR), (F) number of storage roots (#SR), fibrous root fresh weight (FR_{FW}) and storage root fresh weight (SR_{FW}) at final harvest as affected by 60 days of water stress. (●) WS TOL+SUS; (○) WW TOL+SUS. Vertical bars are ±SEM; n = 75 plants per treatment/sampling day


A
PH, cm plant-1
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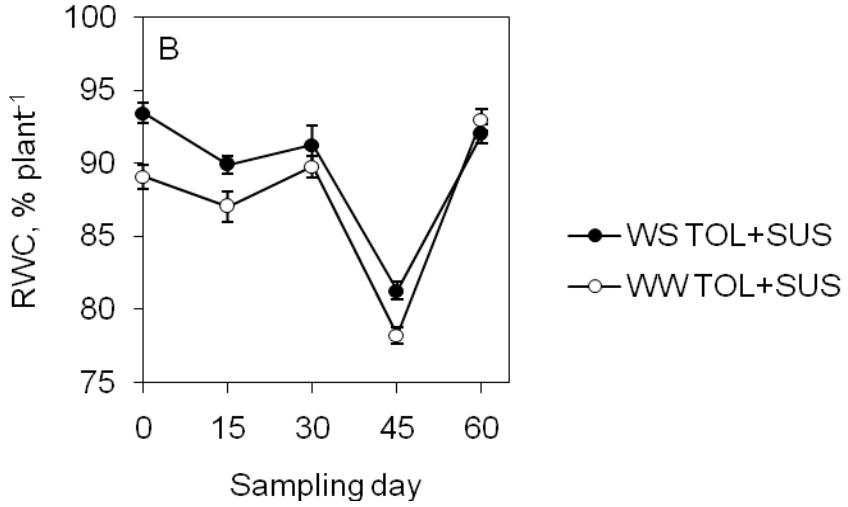
300
250
200
150
100
0
15
30
45
60
Sampling day
WS TOL+SUS
WW TOL+SUS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RWC, % plant-1
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95
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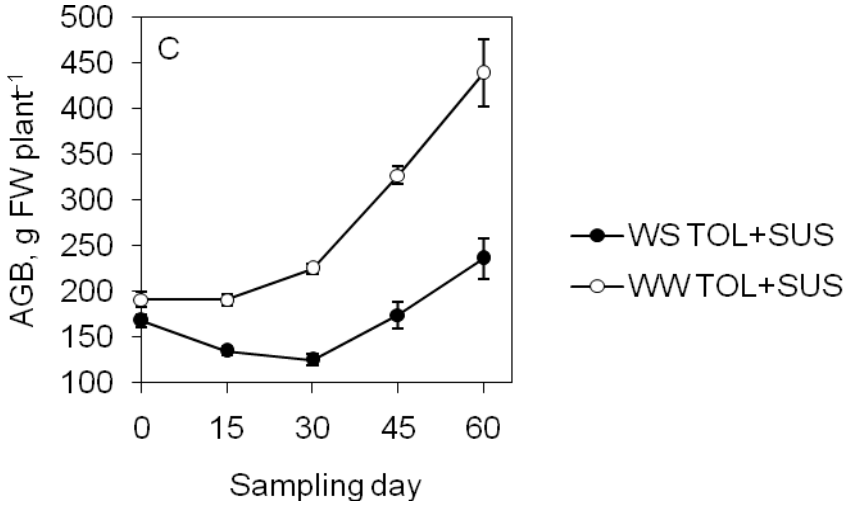
85
80
75
0
15
30
45
60
Sampling day
WS TOL+SUS
WW TOL+SUS


C
AGB, g FW plant-1
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100
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15
30
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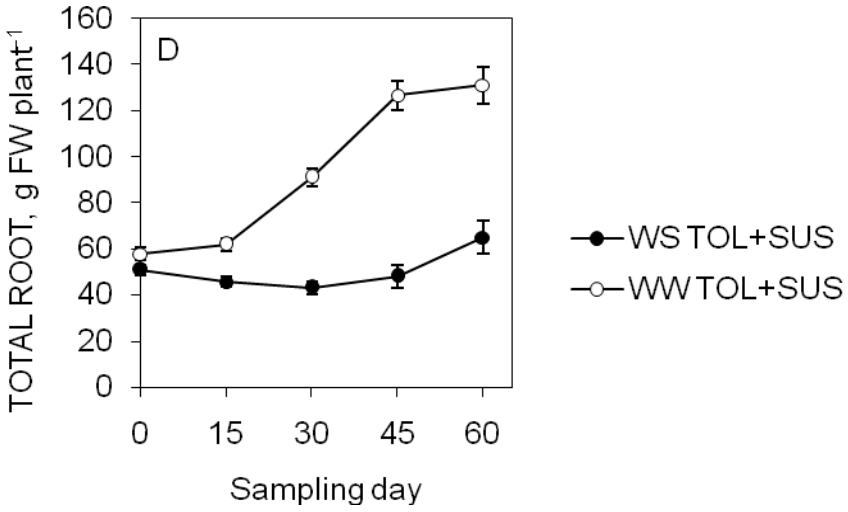
60
Sampling day
WS TOL+SUS
WW TOL+SUS


D
TOTAL ROOT, g FW plant-1
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140
120
100
80
60
40
20
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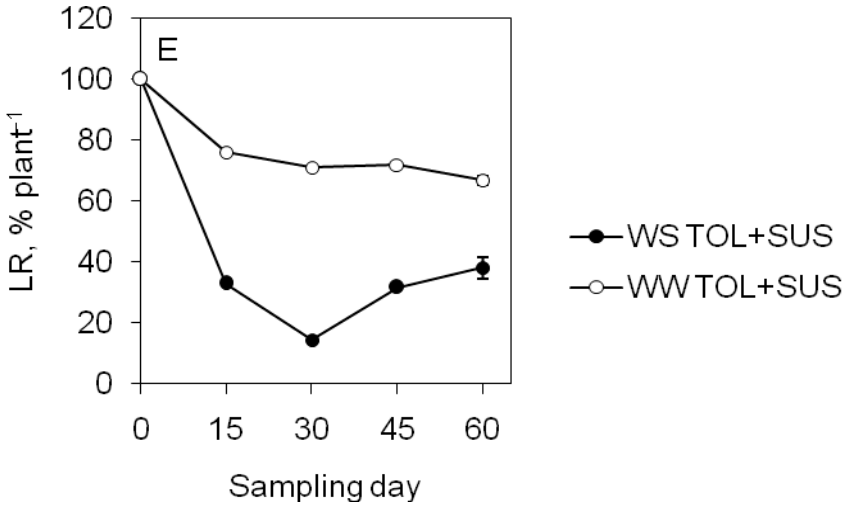
0
15
30
45
60
Sampling day
WS TOL+SUS
WW TOL+SUS


E
LR, % plant-1
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100
80
60
40
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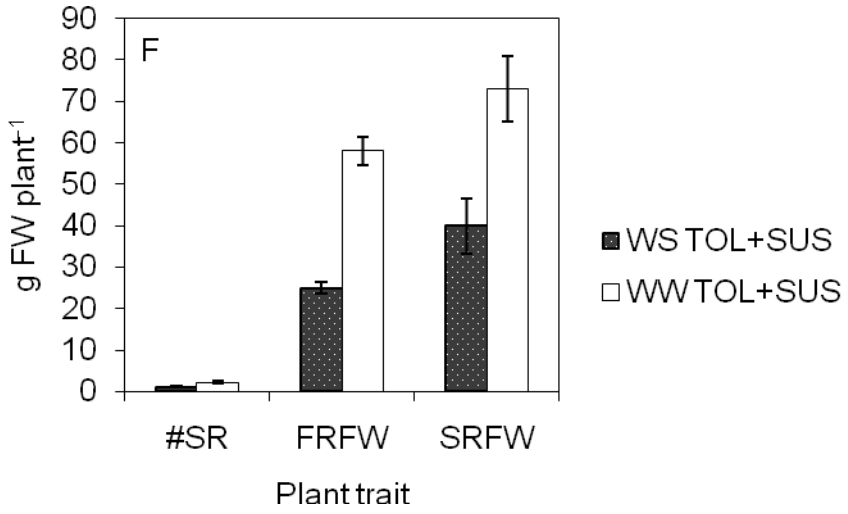
0
0
15
30
45
60
Sampling day
WS TOL+SUS
WW TOL+SUS


F
g FW plant-1
90
80
70
60
50
40
30
20
10
0
#SR
FRFW
SRFW
Plant trait
WS TOL+SUS
WW TOL+SUS

Despite this indication of stress severity and the substantial effects of stress on growth, leaf retention, and other factors, leaf RWC was generally between 85 to 95% and not significantly different between WW and WS treatments throughout the experiment (Figure 4.1B). There was a dip in RWC on DAY 45 due to a severe rise in air temperature coupled with high irradiance within the screen house during the week of the fourth sampling day (DAY 45). This indicates that while water deficit affected growth processes that are highly turgor dependent, the leaves limited their rate of water loss, probably by closing stomata, such that RWC was maintained. In addition, volumetric soil water content (θ) at both shallow (0-5 cm) and deep (25-30 cm) regions was significantly depleted in the WS treatments (Fig. 4.2).

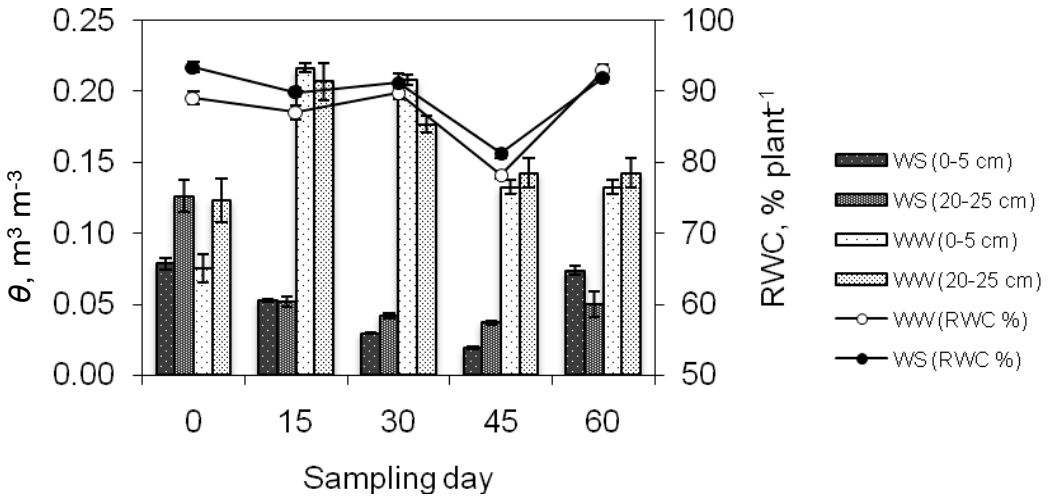
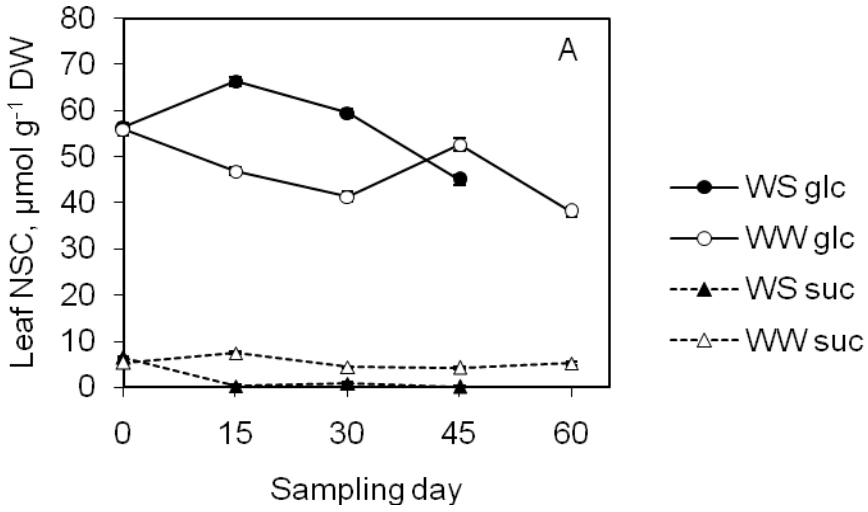


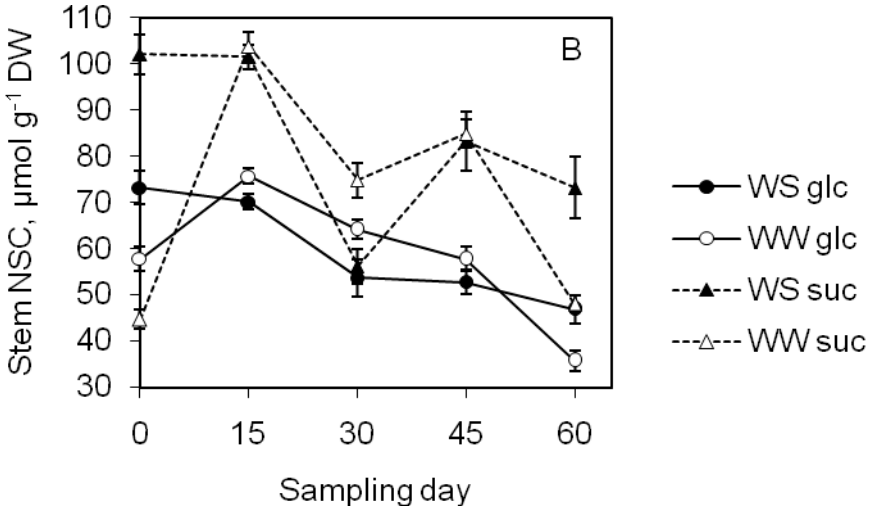
Figure 4.2 Changes in volumetric soil water content (θ) at 0-5 cm and 20-25 cm depth and leaf relative water content (RWC %) under terminal water stress and control conditions over 60 days. Vertical bars are ± SEM; n = 150 plants per sampling day.

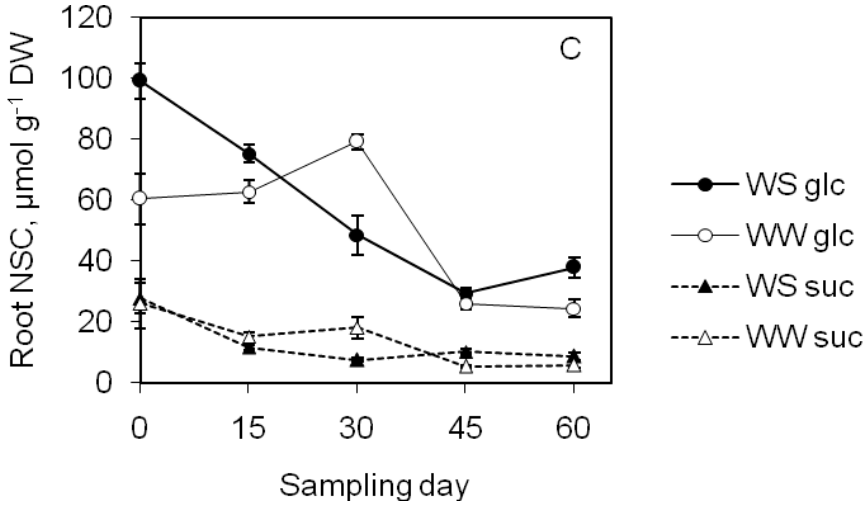
4.3.2 Non-structural carbohydrates dynamics

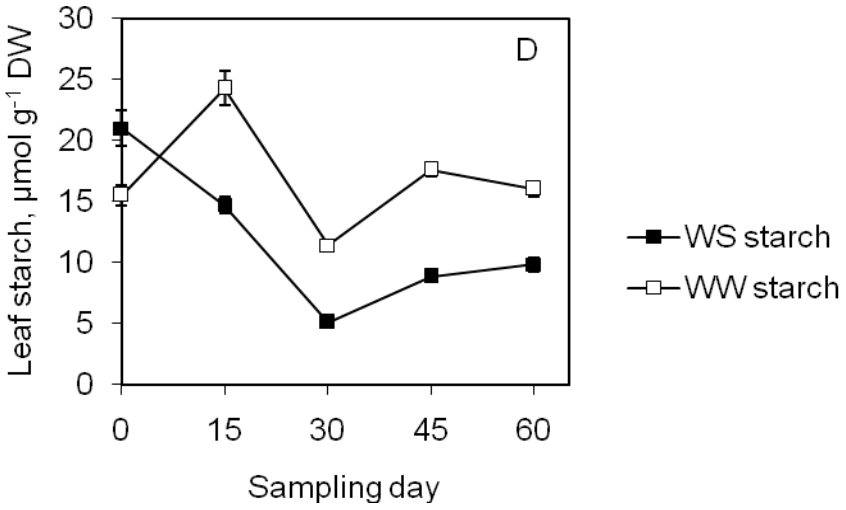
The temporal patterns in non-structural carbohydrates (NSC), including glucose (GLC), sucrose (SUC), and starch (STARCH), were measured in leaves, stems, and roots, both in water stressed (WS) and well-watered (WW) treatments (Fig. 4.3). In general, GLC concentrations were higher in leaves and roots both in WS and WW treatments when compared to SUC concentrations. However, SUC concentrations were higher in stems in both treatments when compared to GLC concentrations (Fig. 4.3A-C). In roots, both GLC and SUC declined during the sampling period in both WW and WS with only a transiently higher level of root GLC in WS than WW at DAY 30 (Fig. 4.3C). Furthermore, leaf starch concentration was lower than leaf GLC and progressively decreased in WS plants from DAY 15 until the end of the experiment (Fig. 4.3A). In stems, GLC and SUC levels were not significantly affected by WS and the levels of sugars were much lower than starch throughout the experiment (Fig. 4.3B and 4.3E). However, stem starch in WW plants accumulated progressively during the experiment to over 2000 umol/g DW (equivalent to 36% w/w of stem biomass) by DAY 45 (Fig. 4.3E). In the WS plants, stem starch accumulated between DAY 0 and DAY 15, then declined gradually during the stress from DAY 15 to DAY 60 (Fig. 4.3E). Root starch increased gradually from DAY 0 to DAY 30 in both treatments, nonetheless at DAY 45 and DAY 60, root starch decreased for both treatments after DAY 30 but presented a small but consistent increase between them by DAY 60 (Fig. 4.3F).

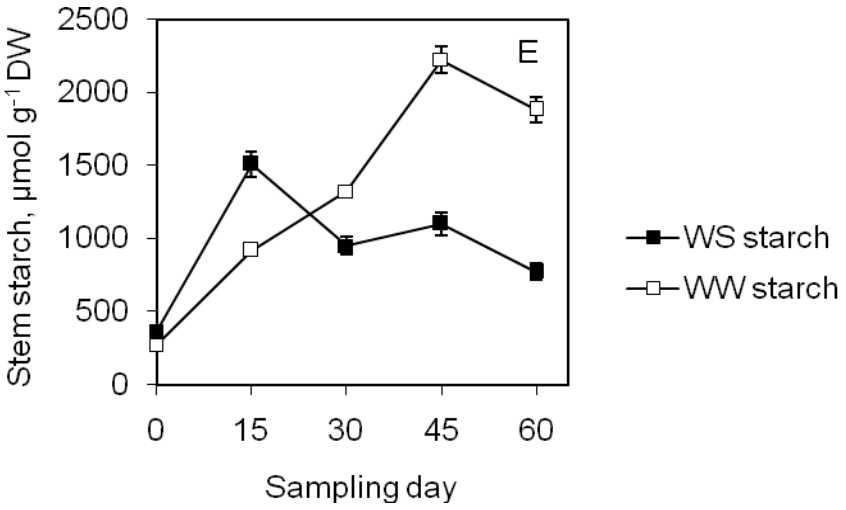
Figure 4.3 Changes in non-structural carbohydrates and starch composition of leaf (A and D), stem (B and E), and root (C and F) sections of cassava plants under terminal water stress and control conditions over 60 days. (●) WS glc, (○) WW glc, (▲) WS suc, (△) WWsuc, (□) WW starch, and (■) WS starch. Vertical bars are ± SEM; n = 75 plants per treatment/sampling day. Starch is expressed in umoles of glucose-equivalents.

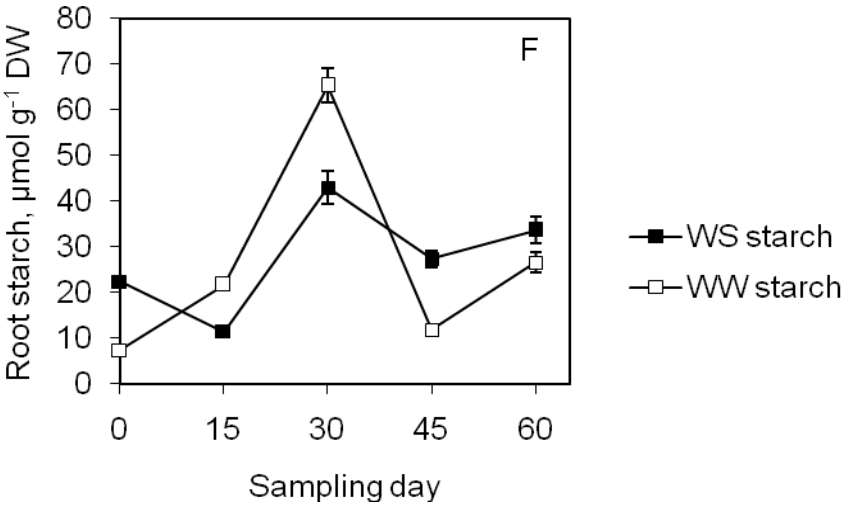

A
Leaf NSC, µmol g-1 DW
80
70
60
50
40
30
20
10
0
0
15
30
45
60
Sampling day
WS glc
WW glc
WS suc
WW suc


B
Stem NSC, µmol g-1 DW
110
100
90
80
70
60
50
40
30
0
15
30
45
60
Sampling day
WS glc
WW glc
WS suc
WW suc


C
Root NSC, µmol g-1 DW
120
100
80
60
40
20
0
0
15
30
45
60
Sampling day
WS glc
WW glc
WS suc
WW suc


D
Leaf starch, µmol g-1 DW
30
25
20
15
10
5
0
0
15
30
45
60
Sampling day
WS starch
WW starch

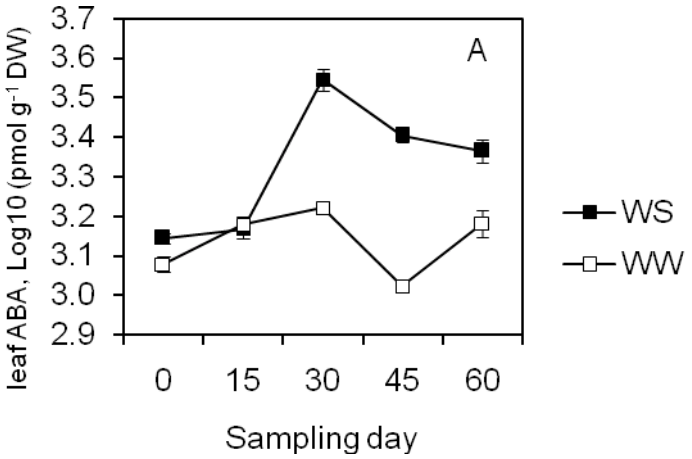

E
Stem starch, µmol g-1 DW
2500
2000
1500
1000
500
0
0
15
30
45
60
Sampling day
WS starch
WW starch

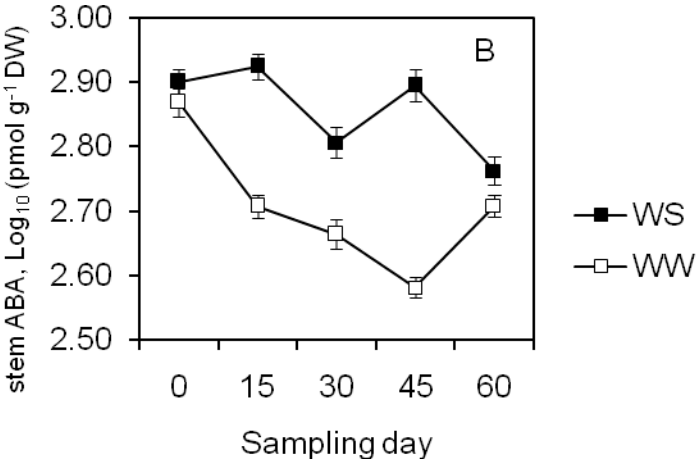

F
Root starch, µmol g-1 DW
80
70
60
50
40
30
20
10
0
0
15
30
45
60
Sampling day
WS starch
WW starch

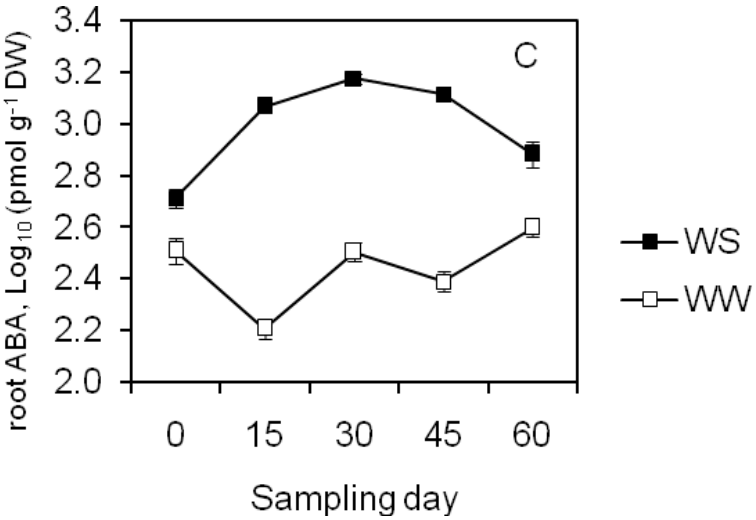
4.3.3 Abscisic acid dynamics

Abscisic acid in leaf, stem, and root tissue (Leaf ABA, Stem ABA and Root ABA, respectively) presented significant differences during water stress (Fig. 4.4). In leaves, ABA was the same in WW and WS at DAY 0 and DAY 15, then at DAY 30 leaf ABA in WS increased to levels substantially higher than WW, and remained high at DAY 45 and DAY 60 (note that the ABA data are presented as the Log_{10} of ABA concentration so that the transformed data is normally distributed and appropriate for statistical analysis) (Fig. 4.4A). The increase in leaf ABA was first observed on DAY 15 and continued until DAY 30 after which a small reduction was evident until DAY 60. In stems, ABA concentration was significantly higher in WS than WW at DAY 15, and remained higher at DAY 30 and DAY 45 before the gap between treatments was closed at DAY 60 (Fig. 4.4B). There was a declining trend in stem ABA concentration in both WW and WS during the experiment, perhaps because starch accumulation in this time frame diluted ABA on a dry weight basis (Fig. 4.3E). In leaves, water deficit increased ABA levels at DAY 30, and they remained significantly higher than WW throughout the remainder of the experiment (Fig 4.4C). Overall, all three tissues of plants subjected to water stress accumulated about two-fold more ABA than WW when expressed on an arithmetic basis (μmol/gDW). Water stress effects on ABA were first observed at DAY 15 in stems and fibrous roots, and at DAY 30 in leaves, and were generally maintained elevated at DAY 30 to DAY 60, consistent with the timing of stress effects on growth and biomass (Fig. 4.1) and carbohydrate levels (Fig. 4.3). This indicates that although water stress had no apparent effect on the relative water contents of leaves throughout the experiment (Fig. 4.1B and Fig. 4.2), it had clear-cut effects on other aspects of plant growth, physiology, and stress hormone levels that indicate the timing and severity of the imposed stress.

Figure 4.4 Changes in abscisic acid (ABA) of (A) leaf, (B) stem, and (C) root sections of cassava plants under terminal water stress and control conditions over 60 days. (■) WS, (□) WW. Vertical bars are ± pooled SEM; n = 75 plants per treatment/sampling day. Units are Log_{10}(pmol g^{-1} DW).


A
leaf ABA, Log10 (pmol g-1 DW)
3.7
3.6
3.5
3.4
3.3
3.2
3.1
3.0
2.9
0
15
30
45
60
Sampling day
WS
WW


B
stem ABA, Log10 (pmol g-1 DW)
3.00
2.90
2.80
2.70
2.60
2.50
0
15
30
45
60
Sampling day
WS
WW


C
root ABA, Log10 (pmol g-1 DW)
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
0
15
30
45
60
Sampling day
WS
WW

4.3.4 Relative yield and its components

On the basis of the temporal pattern of water stress development in the data shown in Figures 4.1 to 4.4, DAY 15 was defined as the initial phase of stress development where only some of the stress-affected traits were impacted, and days 30, 45, and 60 were dates when water stress was at a consistent level of severity, as indicated by ABA and growth data. To compare the genotypes categorized as drought susceptible versus tolerant, the data for WW and WS treatments were analyzed for the time frame during stress from Day 30 to Day 60. As shown in the temporal development data (Fig. 4.1), water stress significantly ($P<0.05$) decreased storage root fresh weight (SR_{FW}), number of storage roots (#SR), plant height (PH), above ground biomass fresh weight (AGB_{FW}), and fibrous root fresh weight (FR_{FW}) when compared to controls (Table 4.2). However, no significant differences were found when drought tolerant and drought susceptible genotypes were compared with each other, except for the number of storage roots. In general, interaction effects between type and treatments were non-significant (Table 4.2).

Table 4.2 The effect of water stress on yield, yield components, and morpho-physiological traits of drought tolerant and susceptible cassava genotypes based on sampling at DAY 30, 45, and 60 except for storage root fresh weight (SR_{FW}), number of storage roots (#SR), fibrous root fresh weight (FR_{FW}), and fresh weight partitioning index (PI), which are based on DAY 60 sampling. Letters refer to differences within traits between treatments. Values followed by different letters are significantly different from each other at the $P<0.05$ level. ***Significant at $P<0.001$; •Significant at $P<0.10$; ns: not significant.

Type	Treatment	RWC	$\theta_{0\text{-}5}$	$\theta_{20\text{-}25}$	PH	AGB_{FW}	SR_{FW}	#SR	FR_{FW}	LR	PI
		%	m^3*m^3	m^3*m^3	cm	g	g		g	%	
Tolerant	WW	86.2 ^{a}	0.18 ^{a}	0.14 ^{a}	246.3 ^{a}	325.5 ^{a}	77.5 ^{a}	2.5 ^{a}	59.6 ^{a}	69.8 ^{a}	0.15 ^{a}
Susceptible	WW	86.4 ^{ab}	0.18 ^{a}	0.15 ^{a}	245.6 ^{a}	318.8 ^{a}	67.7 ^{ab}	1.6 ^{b}	55.7 ^{a}	70.3 ^{a}	0.11 ^{a}
Tolerant	WS	87.7 ^{ab}	0.03 ^{b}	0.04 ^{b}	184.4 ^{b}	184.7 ^{b}	47.8 ^{ab}	1.4 ^{b}	26.3 ^{b}	28.8 ^{b}	0.11 ^{a}
Susceptible	WS	90.5 ^{b}	0.04 ^{b}	0.04 ^{b}	184.2 ^{b}	165.6 ^{b}	25.8 ^{b}	0.6 ^{c}	22.5 ^{b}	26.1 ^{b}	0.08 ^{a}
Type		ns	ns	ns	ns	ns	ns	***	ns	ns	ns
Treatment		***	***	***	***	***	***	***	***	***	•
Interaction		ns	ns	ns	ns	ns	ns	ns	ns	ns	•

4.3.5 Non-structural carbohydrates and abscisic acid

The average carbohydrate and ABA concentrations for the time-frame from DAY 30 to DAY 60 are shown in Table 4.3. Water stress significantly ($P<0.001$) increased ABA levels in leaves, fibrous roots, and stems during stress. However, the extent of ABA accumulation was not different in tolerant compared to susceptible genotypes and there was not an interaction with stress treatment. For carbohydrates, the WS effects were different in the three organs. In both leaves and fibrous roots, WS decreased glucose and sucrose levels, and in leaves it decreased starch levels. These effects are consistent with the expected effect of stress in decreasing photosynthesis and production of carbohydrate.

In leaves, the tolerant genotypes had significantly ($P<0.05$) lower sucrose than susceptible genotypes, but this effect was rather isolated given that less than 12% of the leaf sugar was in sucrose, and the effect was most apparent in the WW treatment. In fibrous roots, sucrose concentration was significantly ($P<0.05$) higher in tolerant than susceptible genotypes in the WS treatment, and in both WW and WS treatments the tolerant genotypes had a significantly ($P<0.05$) higher fraction of sugar in sucrose (FSUC) than susceptible genotypes. In stems, starch was the predominant nonstructural carbohydrate, and WS significantly ($P<0.001$) lowered the levels of starch compared to WW stems. This effect was similar to the effect of WS on sugars in leaves and fibrous roots, and is an expected consequence of WS decreasing photosynthetic production of carbohydrate. However, the data for sugar levels in stems indicated that there were underlying differences in carbohydrate metabolism between tolerant and susceptible genotypes. While stems of WS and WW plants did not differ in sugars, the tolerant genotypes had significantly ($P<0.001$) higher glucose and sucrose levels than susceptible genotypes.

Among the three organs examined (leaf, fibrous roots, and stems), roots and stems had a much higher FSUC than leaves (Table 4.3), with the highest FSUC in stems. Although stems primarily accumulated starch, the FSUC data indicates that metabolism of phloem-imported sucrose in fibrous roots and stems differs in tolerant and susceptible genotypes.

Table 4.3 The effect of water stress on leaf, stem, and root non-structural carbohydrates and abscisic acid on drought tolerant and susceptible cassava genotypes based on sampling at DAY 30, 45 and 60. Letters refer to differences within traits between treatments. Values followed by different letters are significantly different from each other at the *P*<0.05 level. ***Significant at P<0.001; *Significant at *P*<0.05; •Significant at P<0.10; ns: not significant.

		Leaves$_{(L)}$					
Type	Treatment	NSC$_L$				ABA$_L$	*L*ABA$_L$
		GLC$_L$	SUC$_L$	*F*SUC$_L$	STARCH$_L$		(log10)
		µmol g^{-1} DW	µmol g^{-1} DW		µmol g^{-1} DW	pmol g^{-1} DW	pmol g^{-1} DW
Tolerant	WW	44.04 ^{a}	4.11 ^{a}	0.090 ^{a}	14.43 ^{a}	1510.5 ^{a}	3.13 ^{a}
Susceptible	WW	44.23 ^{a}	5.45 ^{b}	0.110 ^{a}	15.38 ^{a}	1540.5 ^{a}	3.15 ^{a}
Tolerant	WS	30.49 ^{b}	0.38 ^{c}	0.005 ^{b}	10.11 ^{b}	2768.4 ^{b}	3.4 ^{b}
Susceptible	WS	30.38 ^{b}	0.38 ^{c}	0.006 ^{b}	7.63 ^{b}	3037.1 ^{b}	3.44 ^{b}
Type		ns	•	ns	ns	ns	ns
Treatment		***	***	***	***	***	***
Interaction		ns	•	ns	•	ns	ns

		Stem$_{(S)}$					
Type	Treatment	NSC$_S$				ABA$_S$	*L*ABA$_S$
		GLC$_S$	SUC$_S$	*F*SUC$_S$	STARCH$_S$		(log10)
		µmol g^{-1} DW	µmol g^{-1} DW		µmol g^{-1} DW	pmol g^{-1} DW	pmol g^{-1} DW
Tolerant	WW	58.16 ^{a}	83.06 ^{a}	0.550 ^{a}	2382.5 ^{a}	485.2 ^{a}	2.64 ^{a}
Susceptible	WW	41.05 ^{a}	61.72 ^{a}	0.560 ^{b}	2253.8 ^{a}	490.3 ^{a}	2.81 ^{a}
Tolerant	WS	57.66 ^{b}	76.06 ^{a}	0.560 ^{b}	1169.1 ^{b}	754.5 ^{b}	2.81 ^{b}
Susceptible	WS	37.93 ^{b}	60.44 ^{a}	0.630 ^{b}	989.3 ^{b}	733.8 ^{b}	2.82 ^{b}
Type		***	***	ns	ns	ns	ns
Treatment		ns	ns	***	***	***	***
Interaction		ns	ns	ns	•	ns	ns

		Root$_{(R)}$					
Type	Treatment	NSC$_R$				ABA$_R$	*L*ABA$_R$
		GLC$_R$	SUC$_R$	*F*SUC$_R$	STARCH$_R$		(log10)
		µmol g^{-1} DW	µmol g^{-1} DW		µmol g^{-1} DW	pmol g^{-1} DW	pmol g^{-1} DW
Tolerant	WW	56.60 ^{a}	6.61 ^{a}	0.189 ^{bc}	35.77 ^{a}	417.6 ^{a}	2.49 ^{a}
Susceptible	WW	51.55 ^{b}	5.08 ^{b}	0.148 ^{c}	31.5 ^{a}	411.4 ^{a}	2.5 ^{a}
Tolerant	WS	39.07 ^{b}	10.44 ^{b}	0.313 ^{a}	33.65 ^{a}	1321.3 ^{b}	3.04 ^{b}
Susceptible	WS	37.45 ^{b}	5.83 ^{b}	0.247 ^{b}	36.06 ^{a}	1407.2 ^{b}	3.08 ^{b}
Type		ns	*	*	ns	ns	ns
Treatment		*	ns	***	ns	***	***
Interaction		ns	ns	ns	ns	ns	ns

4.3.6. Biomass distribution

To examine overall biomass allocation in response to WW and WS treatments, and in drought susceptible and tolerant genotypes, the biomass data for the final harvest on DAY 60 are graphed in Figure 4.4. In susceptible genotypes, WS decreased aboveground biomass fresh weight (AGB_{FW}) (~185 g) relative to WW controls (~415 g) (Δ55%; $P<0.05$), whereas in tolerant genotypes, WS decreased aboveground biomass fresh weight (~262 g) when compared to WW controls (~456 g) (Δ43%; $P<0.05$) . A similar trend was found in root-zone distribution. Water stress decreased storage root fresh weight (SR_{FW}) by 62% ($P<0.05$) in susceptible genotypes and 38% ($P<0.05$) in tolerant genotypes, while in fibrous roots, WS was decreased by 60% ($P<0.05$) in susceptible genotypes and 56% ($P<0.05$) in tolerant. The susceptible and tolerant genotypes also appeared to differ in their partitioning index in response to water stress. Under WS conditions the susceptible genotypes appeared to favor partitioning to fibrous roots; the proportion of fresh biomass in fibrous roots was 9.7% in susceptible genotypes whereas it was 7.9% in tolerant genotypes. In contrast, under WS conditions the storage root partitioning index was 11% in susceptible genotypes and 14.3% in tolerant genotypes. These differences appeared to be specific to the WS treatment. Under WW conditions the partitioning index in storage root fresh weight was about the same in susceptible and tolerant genotypes (12.5 and 13.5%, respectively). Overall, tolerant plants under water stress had a smaller reduction in total dry weight when compared to their susceptible counterparts in all plant parts measured.

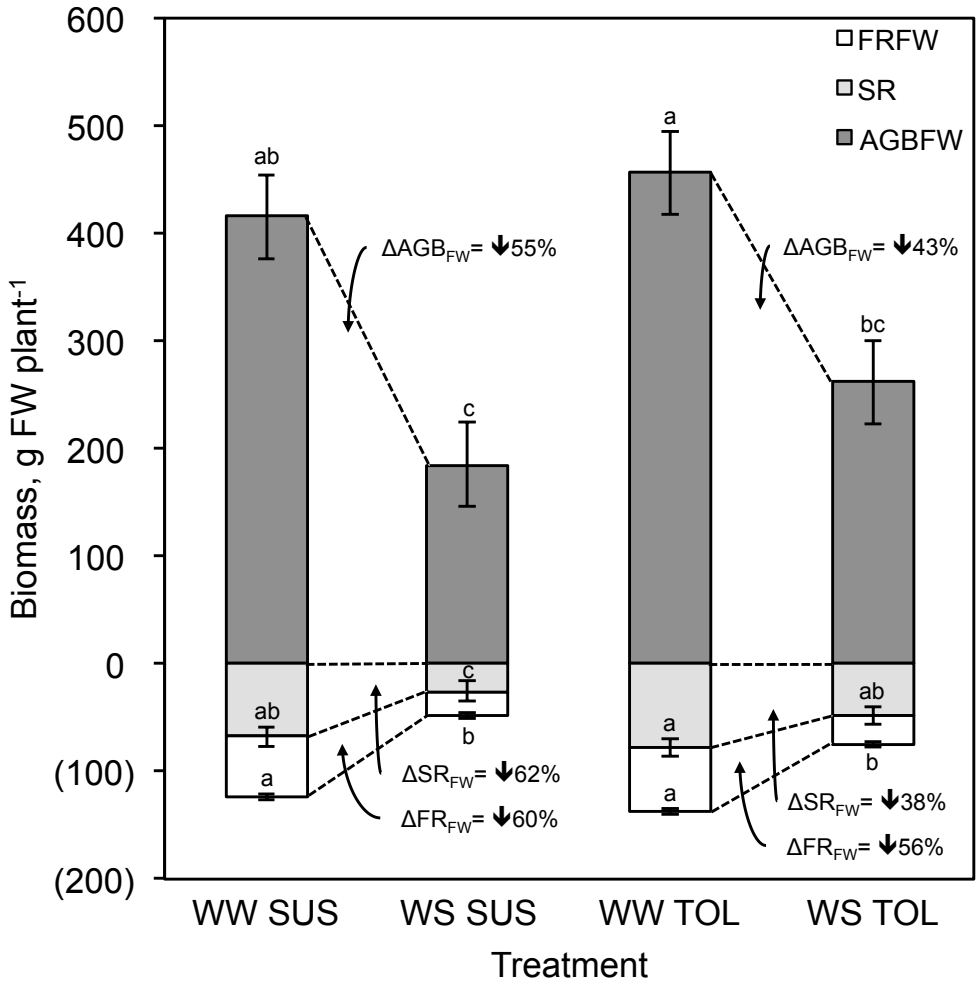


Figure 4.4 Biomass partitioning in well-watered and water-stressed drought susceptible and tolerant cassava genotypes at DAY 60. Decreases in percent change (Δ) are represented outside each column and depict areas within the dotted lines. Percent changes are compared between genotype groups. Vertical bars are ± SEM; n = 150 plants per sampling day.

4.4 Discussion

The objective of this study was to identify cassava traits that are associated with genotypic differences in drought tolerance, and to determine the temporal pattern of the stress responses. The water stress treatment was imposed at a relatively early stage of cassava

development, 60 days after planting the propagation stakes, which coincides with the timing of storage root initiation and early bulking (Deoliveira et al., 1982; Okogbenin and Fregene, 2002). Sampling for the study was conducted during the time course of stress development and early tuber growth so that the timing of responses could be evaluated. Some studies have indicated that cassava storage root development is especially sensitive to drought and photosynthesis-limiting shade stress at this stage, such that storage root yield is severely affected by a relatively short-term stress, whereas stress at later stages of tuber bulking is less damaging to yield (Aresta and Fukai, 1984; Deoliveira et al., 1982). Other studies have indicated that cassava's development is set back by early drought stress, but is capable of recovering (Baker et al., 1989; El-Sharkawy and Cadavid, 2002). El-Sharkawy and Cadavid (2002) found that although early water stress had greater negative impact on aboveground biomass at 12 months than mid-season or terminal stress, storage root yield at 12 months was equally decreased by stress imposed at early, mid-season, or terminal stages. Moreover, in breeding programs it would be valuable to identify genotypes with desirable drought behavior at an early stage of plant growth so that fewer lines would need to be targeted for crossing and further evaluation.

This experiment used two groups of cassava genotypes: one considered "drought-susceptible" and the other "drought-tolerant", defined by cassava breeders from CIAT based on yield trials in different agro-ecological areas throughout Colombia and Brazil. Only a few traits differentiated the two categories of genotypes. At the final harvest date, there was evidence that the tolerant lines have a smaller percent reduction in biomass accumulation in WS relative to their biomass in WW conditions (Fig. 4.4). Also, the partitioning index into storage roots was decreased to a lesser extent in tolerant than susceptible genotypes, and this was associated with a larger number of storage roots initiated in the tolerant genotypes (Table 4.2). Among the traits

assessed in other organs, the tolerant genotypes differed from susceptible in sugar metabolic fractions in fibrous roots and stems, though the trends differed in the two organs. In fibrous roots, the tolerant genotypes had a higher fraction of sugar as sucrose, whereas in stems, the tolerant genotypes had a lower fraction of sugar as sucrose (Table 4.3). In other plant species, water stress has been shown to alter invertase activity such that the fraction of sugar in the hexose versus sucrose fraction is altered. For example, in maize leaves and roots, water stress increases expression of a vacuolar invertase, and this increases the proportion of sugar as hexose (Kim et al., 2000; Trouverie et al., 2004). This could enhance osmotic solute concentration since two moles of hexose are generated for each sucrose hydrolyzed. In contrast, in ovaries of maize, water stress inhibits invertase expression so that more sugar exists as sucrose (Qin et al., 2004). These organ specific responses are thought to reflect the particular developmental process underway during the stress. In the current work, Fsuc was not affected by WS, but it differed, in organ-specific ways, in susceptible compared to tolerant genotypes. In addition, in stems, the tolerant genotypes had higher concentrations of glucose and sucrose than were present in susceptible genotypes (Table 4.3). While a precise interpretation of this finding cannot be made without further information, it suggests that differences in carbohydrate metabolism in stems might be related to the more favorable partitioning of biomass that is found in the tolerant than susceptible genotypes during water stress (Fig. 4.4).

The current study supported cassava's characterization as a drought avoider. Leaf relative water content in the WS treatment remained at values similar to controls throughout the experiment in the full set of 15 diverse genotypes (Fig. 4.1B, Fig. 4.2). Maintenance of RWC occurred while soil water content was depleted (Table 4.2, Fig. 4.2) and growth was inhibited (Fig. 4.1, Table 4.2). This behavior is thought to be caused by acute sensitivity of stomata to

minor decreases in leaf water potential (Ψ_W) during periods of water stress, as has been observed in other species (Damour et al., 2010). This sensitivity is also responsible for cassava's stomatal closure in response to decreases in atmospheric humidity and high transpiration demand in the afternoon (El-Sharkawy and Cock, 1984; Itani et al., 1999). Because of this phenomenon, leaves drastically limit water loss and maintain leaf Ψ_W similar to well-watered controls during extended periods of stress. Maintenance of a relatively high leaf Ψ_W coupled by a high RWC under stress places cassava in the category of plants described as isohydric (Setter and Fregene, 2007; Tardieu and Simonneau, 1998). Although this characteristic is advantageous for maintaining high water status, it can also hinder growth during drought because photosynthesis is restricted to conditions where soil water potential is relatively high (Setter and Fregene, 2007). A hypothesis for carbon starvation and isohydry was described by (McDowell et al., 2008). According to this hypothesis, one would assume that because of cassava's extreme decrease in stomatal conductance and photosynthetic CO_2 assimilation during stress, carbon depletion would pose an additional challenge to cassava's survival during extended periods of drought. In order to cope with this phenomenon, recent studies have indicated that cassava relies heavily on its stored carbohydrate reserves within its stem as well as in its petioles (Duque and Setter, 2005). In the current study, the stems of WW plants accumulated large amounts of starch, equivalent to 36% of stem dry weight at DAY 45 (Fig. 4.3). In the WS plants, starch reserves were high at DAY 15, and gradually decreased during stress, consistent with remobilization and utilization throughout the plant to sustain tissue metabolism and viability. Studies of several crop species have indicated that utilizing stem reserves under stress can improve carbon balance under photosynthate-limiting conditions (Blum, 2005; Reynolds and Tuberosa, 2008). Specifically, in grain crops, soluble carbohydrate reserves in the stem at the time of anthesis may contribute to

superior performance under drought stress (Kumar et al., 2007), and are associated with improved yield potential in field environments where temporary storage helps plants cope with short-term stresses that limit photosynthate supply (Shearman et al., 2005).

Abscisic acid accumulation under stress was also assessed in several cassava organs and compared to controls (Fig 4.4). While changes in leaf RWC could not be detected, ABA increased substantially early in the stress episode (DAY 15) in fibrous roots and stems at the time that inhibition of growth was first observed. Alves and Setter (2000) and Duque and Setter (2005) obtained similar results for leaves in studies of water stressed cassava under controlled greenhouse conditions. This is the first report for cassava of ABA accumulation in fibrous roots and stems. The ABA increases in stems and fibrous roots support the possibility that ABA may play a role in the observed changes in partitioning of growth and carbohydrate metabolism in the genotypes during WS. In other systems, there is evidence ABA affects biomass partitioning and carbohydrate metabolism. For example, increases in root:shoot biomass ratio during WS have been shown to involve differential sensitivity of these two plant parts to ABA (Yamaguchi and Sharp, 2010), and decreases in FSUC in leaves and roots of maize have been associated with ABA-induced expression of vacuolar invertase (Trouverie et al., 2004).

Leaf retention or conversely leaf abscission is a trait that has been extensively studied in cassava (Duque, 2007; Ike and Thurtell, 1981b; Lenis et al., 2006; Ramanujam, 1990). In the current study, leaf retention was significantly decreased by WS, contributing to the overall decrease in accumulation of aboveground biomass. Drought susceptible and tolerant genotypes did not differ in the leaf retention trait. In contrast, a recent study provided evidence that genotypes with greater leaf retention have better yield performance in stress environments (Lenis et al., 2006). In that study, 1350 cassava clones were evaluated in field plots on the North Coast

of Colombia in which plants were subjected to a 5-month dry period towards the end of the growth cycle. In contrast, in the current study, potted plants were subjected to water deficit over a 60-day time-frame during the early phase of plant development. Thus, a possible difference between these two studies is that in field plots a contributor to plant performance and leaf retention might have been genotypic differences in the ability to develop deeper roots and access more soil water, whereas in potted plants such root growth traits are likely to have less effect. It is also possible that the groups of genotypes investigated in the two studies differed in their underlying genetic and physiological characteristics that regulate leaf retention/abscission. Further study is warranted on leaf retention to improve our understanding of this trait and its contribution to drought tolerance.

In the current study, early stress decreased biomass accumulation of all three plant fractions and the partitioning index for storage roots was not detectably affected by WS (Fig. 4.4, Table 4.2). However, drought tolerant genotypes had a higher partitioning index than susceptible genotypes during the stress. Under field conditions with prolonged water stress, it has been observed that cassava produces less total biomass but increases its partitioning index into storage roots (i.e., harvest index) (Connor et al., 1981; El-Sharkawy and Cadavid, 2002; El-Sharkawy et al., 1992a). This has been explained as a consequence of WS inhibition of stem biomass accumulation, leaf abscission, and vigorous leaf regrowth during periods of renewed rainfall. The stress-recovery cycles have the effect of inhibiting stem growth more than leaf and storage root growth. Furthermore, studies have indicated that genotypic differences in storage root yield in drought environments are related to both a genotype's ability for biomass accumulation and its harvest index (El-Sharkawy and Cock, 1990; Lenis et al., 2006). Thus, the observed genotypic differences in storage root partitioning index (Fig. 4.4, Table 4.2), which were measured at an

early stage of development and which are associated with drought tolerance versus susceptibility, suggest that early evaluation of storage root biomass could be an effective method by which cassava genotypes are screened for favorable drought tolerance response.

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CHAPTER 5

5.1 Conclusions and Future Perspectives

The main objectives of this research were to elucidate which of several morphological and physiological traits contribute to cassava's drought tolerance, and evaluate the potential to use these traits in cassava breeding programs.

The developmental mechanisms that allow cassava to tolerate prolonged periods of water deficit can involve numerous attributes (i.e.; morphological, physiological, and biochemical). For example, leaf canopy temperature showed some promise as a way to screen genotypes for early stomatal closure during stress and was mildly correlated with storage root fresh weight. In contrast, leaf retention was poorly correlated with storage root fresh weight under stress, probably due to the fact that genotypes cope with a reduced though sufficient photosynthetic capacity through a small set of leaves near the shoot apex. This capacity is of most importance because cassava can shed lower leaves, reduce leaf area index, lower transpirational area and rate, and still retain some photosynthetically active leaves. In addition, leaf chlorophyll greenness readings were similar in both control and water stressed plants when measured on upper canopy leaves, which suggests that photosynthetic capacity was maintained during water deficit. At the relatively early stage of development when the final harvest was taken in these studies, fibrous root fresh weight was higher in all instances compared to storage root fresh weight under stress. This is consistent with the formation of root systems dedicated to deep-water exploration in field soils. In addition, several correlations between morpho-physiological traits and storage root biomass were found and useful genetic variation (i.e., leaf ABA, leaf

retention, leaf canopy temperature, and partitioning index, among other) exists in cassava germplasm, indicating the potential for further genetic gains. Thus, screening for these traits and incorporating them in newer elite cassava genotypes is potentially valuable in breeding programs aimed towards improved performance in water-limited environments.

Even though cassava is considered a drought tolerant plant, water stress at an early developmental stage can severely affect the plant's growth, with an overall yield penalty at harvest. Thus, there is room for improvement such that understanding the heritability, phenotypic variance, and consistency of relative performance of morpho-physiological traits across environments is valuable for plant breeders and physiologists alike in their pursuit of feasible breeding schemes for dry regions of the world. Regarding this, our study showed that for several of the traits that were studied, broad sense heritability and phenotypic standard deviation were sufficiently high to predict that response to selection would be successful. In addition, the G × E variance, which detracts from heritability, showed that for most traits, the lack of correlation between genotypic performances in the highly contrasting watering environments used in these studies was responsible for G × E effects. This suggests that to improve cassava for drought-prone environments it is valuable to use water-limited environments in phenotypic selection. Several traits such as plant height, leaf canopy temperature, difference in external temperature, leaf chlorophyll greenness, and partitioning index under stress presented predicted heritabilities that exceeded storage root fresh weight. Hence, these measured traits in addition to being important for explaining physiological adaptations to drought tolerance and to an extent yield under stress, were measured accurately and on a high throughput basis. Thus, trait estimates indicate that, in breeding for drought tolerance, selection for them in conjunction with yield can contribute toward identifying lines with improved performance in water-limited environments.

Also, drought screening was performed at an early stage in development, which is repeatable. Thus, utilizing indirect selection criterion for secondary traits for early yield under stress is a potentially useful tool for cassava breeders. Genotype ranking has proved to be a useful and insightful tool to cluster specific genotypes with respect to trait overall performance, both under stress and well-watered conditions. By this method, two elite cassava genotypes, CM 3306-9 and MBRA 165, consistently ranked superior for early yield and partitioning index in both environments. Specifically, these and certain other cassava genotypes apparently have desired combinations of genetic characteristics that are required for good performance under a range of watering environments.

Future studies should consider the interrelationships between food security (using cassava as a model) and water scarcity. The pivotal point here is to enhance maximum productivity under stress complemented with sustainable and sound agricultural practices. Productivity increases under drought stress have been achieved mainly by removing limitations due to other factors, such as by developing disease and insect tolerance, improving soil fertility, and improving weed control; however, with occasional negative impacts to the environment. The current work suggests that there is also potential for improving cassava for physiological and morphological traits that contribute to better tolerance of water-limited environments. To provide the food for future needs there is a choice between using more land for agriculture thereby encroaching on lands that harbor wildlife or using the same land but with crops that use water more efficiently. The latter can hold promising outcomes.

Finally, cassava water stress research can benefit by collaboration and interaction of scientists with expertise in molecular genetics (i.e., marker assisted selection for drought tolerance), climate change (i.e., weather assessments and modeling), phenotyping (i.e., for

secondary traits or indirect selection), and agricultural management (i.e., conservation agriculture, sustainable practices and eco-friendly agriculture).